CHEMICAL LIBRARY

VOL. IV JULY, 1917 No. 1

AUG 13 1917

# SOIL SCIENCE

FOUNDED BY

## RUTGERS COLLEGE

NEW BRUNSWICK, N. J.

JACOB G. LIPMAN, Editor-in-Chief

ASSISTANT EDITORS

NICHOLAS KOPELOFF CARL R. WOODWARD

IN CONSULTATION WITH

DR. F. J. ALWAY
University of Minnesota, St. Paul, Minn.
DR. C. BARTHEL
Centralanstalten för Försöksväsendet på Jordbruksområdet, Stockholm, Sweden
DR. M. W. BEIJERINCK
Technische-Hoogeschool, Delft, Holland
PROF. A. W. BLAIR
Rutgers College, New Brunswick, N. J.
DR. P. E. BROWN
Iowa State College of Agriculture, Ames, Iowa
DIRECTOR H. R. CHRISTENSEN
Statens Planteavls-Laboratorium, Copenhagen, Denmark
DR. H. J. CONN
New York State Experiment Station, Geneva, N. Y.
DR. H. VON FEILITZEN
Svenska Mosskulturföreningen, Jönköping, Sweden
DR. E. B. FRED
University of Wisconsin, Madison, Wis.
DR. R. GREIG-SMITH
Linnean Society, Sydney, New South Wales
DR. B. L. HARTWELL
Rhode Island Experiment Station, Kingston, R. I.
DR. C. B. LIPMAN
University of California, Berkeley, Calif.
DR. F. LÖHNIS
U. S. Department of Agriculture, Washington, D. C.
DR. T. L. LYON
Cornell University, Ithaca, N. Y.
DR. E. A. MITSCHERLICH
University of Königsberg, Prussia
PROF. C. A. MOOERS
Tennessee Experiment Station, Knoxville, Tenn.
DR. THEO. REMY
Instut für Böden-u Pflanzenbaulehre, Bonn a. Rhein
PROF. G. ROSSI
Royal Agricultural High School in Portici, Naples, Italy
DR. E. J. RUSSELL
Rothamsted Experiment Station, Harpenden, England
DR. O. SCHREINER
U. S. Department of Agriculture, Washington, D. C.
DR. A. A. F. DE SIGMUND
Technische-Hochschule, Budapest, Hungary
PROF. CHAS. E. THORNE
Ohio Experiment Station, Wooster, Ohio
DR. N. TULAIKOFF
Forest Institute, Petrograd, Russia

PUBLISHED MONTHLY BY
WILLIAMS & WILKINS COMPANY
BALTIMORE, MD., U. S. A.

THE CAMBRIDGE UNIVERSITY PRESS
FETTER LANE, LONDON, E. C.

Application has been made for entry as second-class matter at the Post Office at Baltimore, Md., under the act of March 3, 1879
Copyright 1917, by Williams & Wilkins Company

SOIL SCIENCE

Contents for July, 1917

H. L. FULMER. The Relation of Green Manures to Nitrogen Fixation..... 1

NICHOLAS KOPELOFF. The Influence of Fineness of Division of Pulverized Limestone on Crop Yield as well as the Chemical and Bacteriological Factors in Soil Fertility..... 19

J. G. LIPMAN AND A. W. BLAIR. The Yield and Nitrogen Content of Soybeans as Influenced by Lime..... 71

J. W. AMES AND T. E. RICHMOND. Fermentation of Manure Treated with Sulfur and Sulfates: Changes in Nitrogen and Phosphords Content..... 79

---


# SOIL SCIENCE

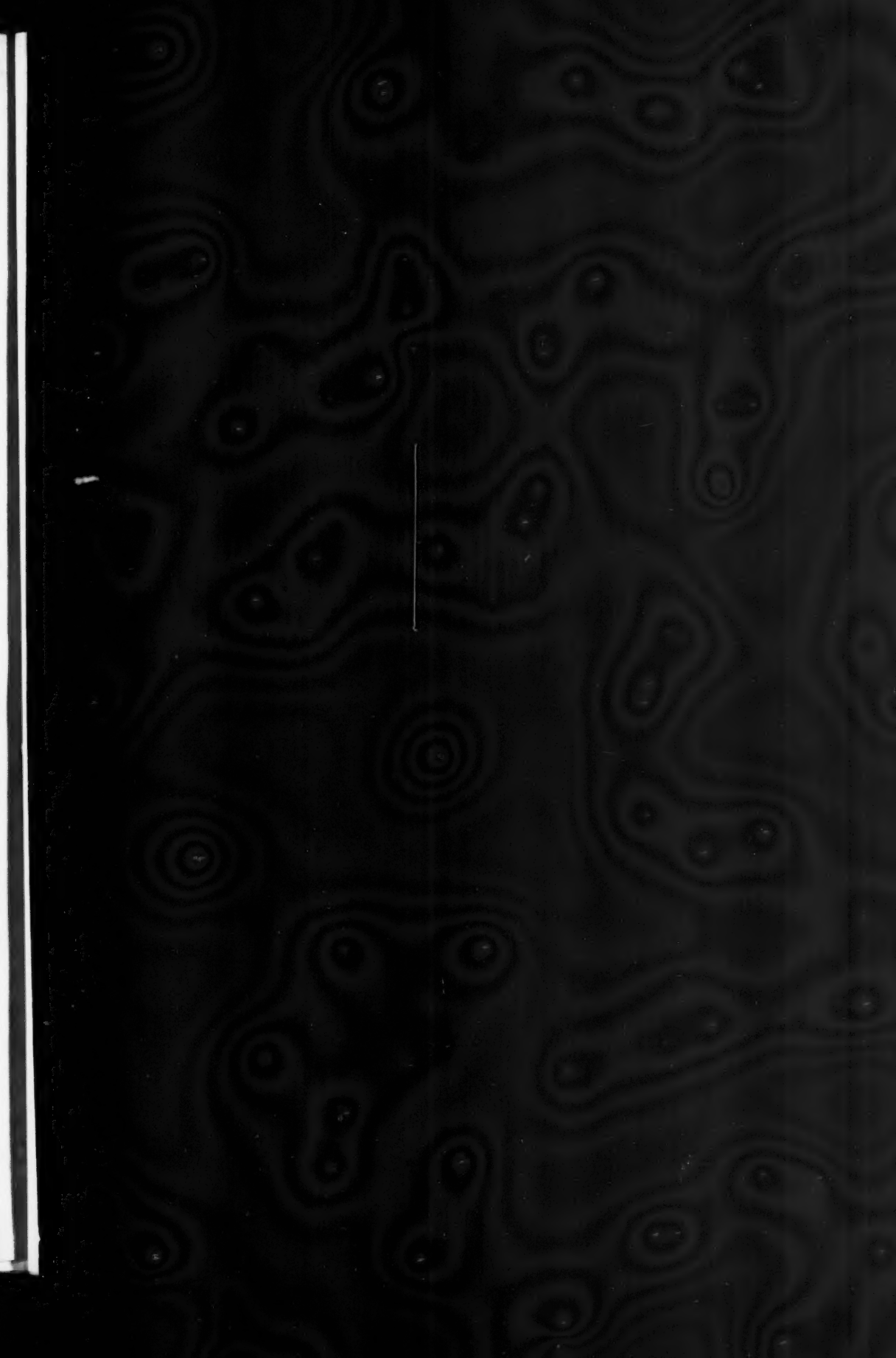
# PRICES

| | | U. S. and Dependencies | Canada | Foreign |
|---|---|---|---|---|
| Volume IV (Numbers 1-6 inclusive)<br>Volume V (Numbers 1-6 inclusive) | (July 1917—June 1918) | $4.00 | $4.25 | $4.50 |
| Volume III (January—June, 1917) | | 3.00 | 3.15 | 3.25 |
| Volume II (July—December, 1916) | | 3.00 | 3.15 | 3.25 |
| Volume I (January—June, [redacted] 1916) | | 3.00 | 3.15 | 3.25 |

**Order today from**

## WILLIAMS & WILKINS COMPANY

## BALTIMORE, U. S. A.



## CONTENTS


| | PAGE |
|---|---|
| The Relation of Green Manures to Nitrogen Fixation. H. L. Fulmer | 1 |
| The Influence of Fineness of Division of Pulverized Limestone on Crop Yield as well as the Chemical and Bacteriological Factors in Soil Fertility. Nicholas Kopeloff | 19 |
| The Yield and Nitrogen Content of Soybeans as Influenced by Lime. J. G. Lipman and A. W. Blair | 71 |
| Fermentation of Manure Treated with Sulfur and Sulfates: Changes in Nitrogen and Phosphorus Content. J. W. Ames and T. E. Richmond | 79 |
| A Preliminary Soil Census of Alabama and West Florida. Roland M. Harper | 91 |
| A Soil Sampler for Bacteriological and Chemical Purposes. J. R. Neller | 109 |
| Experiments in Methods for Determining the Reaction of Soils. Harald R. Christensen | 115 |
| Some Factors Influencing the Quantitative Determination of Nitric Nitrogen in the Soil. J. E. Greaves and C. T. Hirst | 179 |
| The Effects of Alkali Salts on Nitrification. P. E. Brown and E. B. Hitchcock | 207 |
| The Divergent Effects of Lime and Magnesia upon the Conservation of Soil Sulfur. W. H. MacIntire, L. G. Willis and W. A. Holding | 231 |
| The Rate of Water Movement in Aerated Soils. Howard E. Pulling | 239 |
| The Production of Available Phosphorus fom Rock Phosphate by Composting with Sulfur and Manure. P. E. Brown and H. W. Warner | 269 |
| Total Nitrogen and Carbon in Cultivated Land and Land Abandoned to Grass and Weeds. A. W. Blair and H. C. McLean | 283 |
| The Determination of Soil Phosphorus. Clayton O. Rost | 295 |
| Hydrogen-ion Concentration Measurements of Soils of Two Types: Caribou Loam and Washburn Loam. Louis J. Gillespie and Lewis A. Hurst | 311 |
| Further Studies of the Nature of Ammonification. K. Miyake | 323 |
| Nitrification as a Measure of the Availability of Different Forms of Calcium Carbonate when Employed as Correctors of Soil Acidity. P. S. Burgess | 327 |
| Vegetation Experiments on the Availability of Treated Phosphates. Jacob G. Lipman and Harry C. McLean | 337 |
| The Influence of Sodium Nitrate upon Nitrogen Transformations in Soils with Special Reference to its Availability and that of Other Nitrogenous Manures. David A. Coleman | 345 |
| A Correlation between Bacterial Activity and Lime Requirement of Soils. Firman E. Bear | 433 |
| The Moisture Equivalent Determinations of Salt-treated Soils and their Relation to Changes in the Interior Surfaces. L. T. Sharp and D. D. Waynick | 463 |
| Relation of the Mechanical Analysis to the Moisture Equivalent of Soils. Alfred Smith | 471 |
| The Volumetric Determination of Sulfates in Water Extracts of Soils. A. W. Christie and J. C. Martin | 477 |
| A Study of the Root-Nematode (Heterodera Radicicola) and its Control. Willis P. Duruz | 481 |
| Index | 493 |




323851

# ILLUSTRATIONS

## PLATES

PAGE

THE INFLUENCE OF FINENESS OF DIVISION OF PULVERIZED LIMESTONE ON CROP YIELD AS WELL AS THE CHEMICAL AND BACTERIOLOGICAL FACTORS IN SOIL FERTILITY

PLATE I. Galvanized iron pots permitting collection of drainage water, cropped to barley ..... 68

A SOIL SAMPLER FOR BACTERIOLOGICAL AND CHEMICAL PURPOSES

PLATE I. Fig. 1. Apparatus in position for taking the surface section. Fig. 2. Showing the relative size of the tubes ..... 112

SOME FACTORS INFLUENCING THE QUANTITATIVE DETERMINATION OF NITRIC NITROGEN IN THE SOIL

PLATE I. Apparatus used in experiment shown in operation ..... 204

THE DIVERGENT EFFECTS OF LIME AND MAGNESIA UPON THE CONSERVATION OF SOIL SULFUR

PLATE I. Fig. 1. Outside view of hillside lysimeter equipment. Fig. 2. Inside view of hillside lysimeter equipment ..... 236

VEGETATION EXPERIMENTS ON THE AVAILABILITY OF TREATED PHOSPHATES

PLATE I. Pot 2, no phosphorus; pot 4, floats; pot 6, compost; pot 8, commercial acid phosphate ..... 342

A STUDY OF THE ROOT-NEMATODE (HETERODERA RADICICOLA) AND ITS CONTROL

PLATE I. Figs. 1 to 6. Development of free-living nematode. Fig. 7. Young larva. Fig. 8. Immature female. Fig. 9. Mature female. Fig. 10. Mature male ..... 492

## TEXT FIGURES

THE RELATION OF GREEN MANURES TO NITROGEN FIXATION

FIG. 1. Milligrams of nitrogen fixed in 100 grams of garden soil as a result of adding green manure ..... 7

2. Milligrams of nitrogen fixed in 100 grams of field soil as a result of adding green manure ..... 9

3. Milligrams of nitrogen fixed in 100 grams of field soil as a result of adding green manure to soil additionally treated with mannit ..... 11

4. Milligrams of nitrogen fixed in 100 cc. of Ashby's solution inoculated with treated and untreated soil ..... 13

# ILLUSTRATIONS

## PLATES

PAGE

THE INFLUENCE OF FINENESS OF DIVISION OF PULVERIZED LIMESTONE ON CROP YIELD AS WELL AS THE CHEMICAL AND BACTERIOLOGICAL FACTORS IN SOIL FERTILITY

PLATE I. Galvanized iron pots permitting collection of drainage water, cropped to barley ..... 68

A SOIL SAMPLER FOR BACTERIOLOGICAL AND CHEMICAL PURPOSES

PLATE I. Fig. 1. Apparatus in position for taking the surface section. Fig. 2. Showing the relative size of the tubes ..... 112

SOME FACTORS INFLUENCING THE QUANTITATIVE DETERMINATION OF NITRIC NITROGEN IN THE SOIL

PLATE I. Apparatus used in experiment shown in operation ..... 204

THE DIVERGENT EFFECTS OF LIME AND MAGNESIA UPON THE CONSERVATION OF SOIL SULFUR

PLATE I. Fig. 1. Outside view of hillside lysimeter equipment. Fig. 2. Inside view of hillside lysimeter equipment ..... 236

VEGETATION EXPERIMENTS ON THE AVAILABILITY OF TREATED PHOSPHATES

PLATE I. Pot 2, no phosphorus; pot 4, floats; pot 6, compost; pot 8, commercial acid phosphate ..... 342

A STUDY OF THE ROOT-NEMATODE (HETERODERA RADICICOLA) AND ITS CONTROL

PLATE I. Figs. 1 to 6. Development of free-living nematode. Fig. 7. Young larva. Fig. 8. Immature female. Fig. 9. Mature female. Fig. 10. Mature male ..... 492

## TEXT FIGURES

THE RELATION OF GREEN MANURES TO NITROGEN FIXATION

FIG. 1. Milligrams of nitrogen fixed in 100 grams of garden soil as a result of adding green manure ..... 7

2. Milligrams of nitrogen fixed in 100 grams of field soil as a result of adding green manure ..... 9

3. Milligrams of nitrogen fixed in 100 grams of field soil as a result of adding green manure to soil additionally treated with mannit ..... 11

4. Milligrams of nitrogen fixed in 100 cc. of Ashby's solution inoculated with treated and untreated soil ..... 13

PAGE

THE INFLUENCE OF FINENESS OF DIVISION OF PULVERIZED LIMESTONE ON CROP YIELD AS WELL AS THE CHEMICAL AND BACTERIOLOGICAL FACTORS IN SOIL FERTILITY

FIG. 1. Diagram showing the effect of different degrees of nitrogen, in grams, and the decrease in lime-requirement of several soils, expressed in per cent..... 30
2. Diagram of apparatus used in determining carbon dioxide in soil..... 32

A PRELIMINARY SOIL CENSUS OF ALABAMA AND WEST FLORIDA

FIG. 1. Map showing the regions described herein, and also the principal rivers and cities, to illustrate their relations to some of the geographical boundaries... 93

A SOIL SAMPLER FOR BACTERIOLOGICAL AND CHEMICAL PURPOSES

FIG. 1. Longitudinal sections of soil sampler..... 110

EXPERIMENTS IN METHODS FOR DETERMINING THE REACTION OF SOILS

FIG. 1. Diagram showing the influence of the amount of soil and the reaction-time on the amount of acid freed from calcium acetate solution..... 125
2. Diagram showing the influence of the reaction-time on the ability of various soils to free acid from a calcium acetate solution..... 134
3. Apparatus for determining the acidity of the soil according to the Tacke-Süchting method..... 139
4. Diagram showing the relation between the reaction of the soil for litmus and its ability to free acid..... 170

SOME FACTORS INFLUENCING THE QUANTITATIVE DETERMINATION OF NITRIC NITROGEN IN THE SOIL

FIG. 1. Curves showing per cent of nitric nitrogen recovered in sample of soil treated by different antiseptics after varying lengths of time..... 193

THE EFFECTS OF ALKALI SALTS ON NITRIFICATION

FIG. 1. Effects of NaCl on nitrification in normal soil..... 210
2. Effects of $Na_2SO_4$ on nitrification in normal soil..... 211
3. Effects of $MgSO_4$ on nitrification in normal soil..... 212
4. Effects of $CaCO_3$ on nitrification in normal soil..... 214
5. Effects of $NaHCO_3$ on nitrification in alkali soil..... 215
6. Effects of $Na_2CO_3$ on nitrification in alkali soil..... 216
7. Effects of $CaSO_4$ on nitrification in alkali soil..... 218
8. Effects of $CaCO_3$ on nitrification in alkali soil..... 219
9. Effects of $CaCO_3$ and $NaHCO_3$, $Na_2CO_3$ and $CaSO_4$ in the presence of $CaCO_3$ on nitrification in alkali soil..... 221
10. Effects of NaCl, $Na_2SO_4$, $MgSO_4$ and $CaCO_3$ on nitrification in normal soil—Second sampling (pot experiments)..... 223
11. Effects of NaCl, $Na_2SO_4$, $MgSO_4$, and $CaCO_3$ on nitrification in normal soil—Third and fourth samplings (pot experiments)..... 223
12. Effects of $CaCO_3$, $NaHCO_3$, $Na_2CO_3$, and $CaSO_4$ on nitrification in alkali soil—First and second samplings (pot experiments)..... 224
13. Effects of $CaCO_3$, $NaHCO_3$, $Na_2CO_3$, and $CaSO_4$ on nitrification in alkali soil—Third and fourth samplings (pot experiments)..... 224
14. Effects upon beans grown in normal soil with the addition of various alkali salts 227

THE RATE OF WATER MOVEMENT IN AERATED SOILS

PAGE

FIG. 1. The osmometer ..... 241
2. Hypothetical section through a drained soil, showing wedges and films ..... 243
3. Test 1 of the osmometer operating against garden soil ..... 251
4. Test 2 of the osmometer operating against garden soil ..... 252
5. Test 3 of the osmometer operating against garden soil ..... 252
6. Test 4 of the osmometer operating against garden soil ..... 253
7. Test 5 of the osmometer operating against garden soil ..... 253
8. Test 6 of the osmometer operating against garden soil ..... 255
9. Test 7 of the osmometer operating against garden soil ..... 255
10. Test 8 of the osmometer operating against sand ..... 257
11. Test 9 of the osmometer operating against sand ..... 257
12. Initial rates of the soil tests ..... 261
13. Instrument for obtaining soil samples of known field volume ..... 264

THE PRODUCTION OF AVAILABLE PHOSPHORUS FROM ROCK PHOSPHATE BY COMPOSTING WITH SULFUR AND MANURE

FIG. 1. Diagram showing determinations of available phosphorus when compost was used ..... 273
2. Diagram showing determinations of available phosphorus when cow manure was used ..... 275
3. Diagram showing determinations of available phosphorus when horse manure was used ..... 277

NITRIFICATION AS A MEASURE OF THE AVAILABILITY OF DIFFERENT FORMS OF CALCIUM CARBONATE WHEN EMPLOYED AS CORRECTORS OF SOIL ACIDITY

FIG. 1. Curves showing effect of coral sand and finely-ground coral limestone on nitrification ..... 332

THE INFLUENCE OF SODIUM NITRATE UPON NITROGEN TRANSFORMATIONS IN SOILS WITH SPECIAL REFERENCE TO ITS AVAILABILITY AND THAT OF OTHER NITROGENOUS MANURES

FIG. 1. Diagram showing the proportions of the three fertilizers, $NaNO_3$, Acid phosphate, and KCl ..... 382

A CORRELATION BETWEEN BACTERIAL ACTIVITY AND LIME REQUIREMENT OF SOILS

FIG. 1. The effect of calcium carbonate on the number of bacteria in soils I and II .... 457
2. The effect of calcium carbonate on the rate of ammonification in soils I and II with phosphorus ..... 458
3. The effect of calcium carbonate on the rate of nitrification in soils I and II with phosphorus ..... 459
4. The effect of calcium carbonate on non-symbiotic nitrogen fixation in soil II .. 460

THE MOISTURE EQUIVALENT DETERMINATIONS OF SALT-TREATED SOILS AND THEIR RELATION TO CHANGES IN THE INTERIOR SURFACES

FIG. 1. ..... 468

## ERRATA

Page 253, legend for figure 7, "Osmeter" should read "Osmometer."
Page 296, third line above footnote, "exacctly" should read "exactly."
Page 299, table 3, part 1, Average for sample A, "0.038" should read "0.030."
Page 307, table 10, title, "alum num" should read "aluminum."
Page 307, four lines at foot of page should be inserted between ninth and tenth lines from foot of page 308.
Page 413, table 25, first line of figures, "0" should read "3."
Page 420, table 31, second line of figures, "2.77" should read "6.77."

# THE RELATION OF GREEN MANURES TO NITROGEN FIXATION[1]

H. L. FULMER

*From the Agricultural Experiment Station of the University of Wisconsin*

Received for publication March 12, 1917

## INTRODUCTION

The lack of nitrogen and organic matter in many cultivated soils is noted by a decrease in the crop yield. This deficiency is most apparent in the older farmed soils where cultivation and removal of crops has been practiced for a number of years. In order that crop production may be increased, it is important to study the most economical method for supplying nitrogen and organic matter. The turning under of green manures offers a possible solution. Aside from the plant-food returned with the green manure, it frequently benefits the physical and biological properties of the soil. The total number of soil microorganisms, as well as the different groups of microorganisms, is markedly influenced by the addition of green manure. This effect on the soil flora is reflected in the fertilizing constituents made available for plant growth.

Many investigations have been directed toward this phase of the problem and much valuable information has been obtained concerning the relation of green manures to biologic processes in soil. From a review of the literature, it appears that the effect of green manures on nitrogen fixation deserves further study. Accordingly, experiments were planned to investigate the relation of green plant tissue to free nitrogen-fixing organisms. The nature of the problem may be seen from the following brief review of the literature.

## HISTORICAL

Berthelot (2) was the first to note a gain of nitrogen through the action of biological agents on the humus of the soil. In 1902 Henry (9) observed an increase in nitrogen when forest leaves were allowed to decompose in soil. His results were confirmed by Süchting (25) and Hornberger (11).

Koch (15) found a 10 per cent increase in total nitrogen of soil which had been allowed to stand for $2\frac{1}{4}$ years. This increase he attributed to the assimilation of nitrogen by the non-symbiotic nitrogen-fixing organisms. Krzemieniewski (18) studied the gain in nitrogen with pure cultures of *Azotobacter chroococcum*, *Radiobacter*, and *Granulobacter* in solutions containing soil

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

humate. All cultures showed an increase in nitrogen from the use of humate, especially when the first two organisms were grown together. Remy and Rösing (23) obtained similar results with the use of soil humate. They concluded that the iron contained in the humate probably acted as a stimulant for the nitrogen-fixing organisms.

In a sandy soil, Koch and his associates (16) obtained nitrogen fixation by the addition of dextrose, cane sugar, or starch. There was practically no increase shown when straw, filter paper, or buckwheat was applied. The addition of green mustard in a dried, pulverized form resulted in a loss of nitrogen, which loss, they thought, was due partly to the escape of ammonia from the decomposing mustard.

Koch (13) showed that the beneficial effect of sugar, i.e., gain in nitrogen, may be noted by an increase in crop yield. During the first year no increase was noted; however, in the second and third years the treated series showed an increase in crop yield. In a later publication (14) the same author noted that cellulose may be used as a source of energy for nitrogen-fixing organisms provided the time of incubation is long enough for the cellulose to decompose. The greatest gain in nitrogen occurred where the largest amount of cellulose was digested. For each gram of carbohydrate consumed, cellulose gave a greater fixation than dextrose.

According to Hutchinson (12) winter applications of sugar and starch benefited the crop yield. However, spring applications failed to give an increase. He attributed this difference to the effect of the season on *Azotobacter*. Hoffmann (10) found an increase of nearly 1000 pounds of nitrogen per acre as a result of three years' treatment of soil with sugar. Similar results were obtained with starch, but the gain was not so marked. In 1910, Marr (20) reported that straw alone when added to soil increased nitrogen fixation; sugar, also, favored this process.

Experiments carried out by Pringsheim (22) showed that the nitrogen-assimilating organisms can make use of decomposing cellulose as a source of energy. Recent investigations by Bottomley (3) have shown that soluble humates may be used by the nitrogen-assimilating organisms.

Hanzawa (8) employed humus from stable manure and from green manure as energy sources for *Azotobacter*. In solution he noted that stable-manure humus increased nitrogen fixation, while green-manure humus proved injurious. However, only a very limited number of determinations were made with green manures.

Löhnis and Green (19) carried on similar experiments. They worked with mixed cultures of *Azotobacter chroococcum*, *Beijerinckii*, *Vinelandii*, and *Vitreum*. Beijerinck's mannit solution[2] was used with the addition of about 0.2 gm. of dried material to each 100 cc. portion of liquid. They compared the gain in nitrogen with stable manure, green (pea) manure, peat, and straw. Their results are given in the following table:

[2] Tap water, 1,000 cc.; mannit, 20.0 grams; dipotassium phosphate, 0.5 grams.

| MATERIAL | NITROGEN ASSIMILATED IN 100 CC. OF SOLUTION AFTER THREE WEEKS |
|---|---|
| | *mgm.* |
| Fresh straw | 10.0 |
| Fresh stable manure | 9.8 |
| Fresh peat | 9.3 |
| Green manure | 8.0 |
| Beijerinck's mannit solution alone | 5.6 |

The results show that all of the organic substances, when added to the mannit solution, aided nitrogen fixation. Apparently straw, stable manure, and peat furnished the best sources of energy for the nitrogen-fixing organisms. These investigators stated that more nitrogen was assimilated by the mixed cultures than by any one of the individual strains.

When the stable manure, green manure, peat, and straw were converted into humus under aerobic or anaerobic conditions, the effect on nitrogen fixation was as follows:

| MATERIAL | CONDITIONS FOR HUMUS FORMATION | NITROGEN ASSIMILATED IN 100 CC. OF SOLUTION AFTER THREE WEEKS |
|---|---|---|
| | | *mgm.* |
| Stable manure | Aerobic | 14.4 |
| | Anaerobic | 13.6 |
| Green manure | Aerobic | 9.0 |
| Peat | Aerobic | 8.7 |
| | Anaerobic | 9.9 |
| Straw | Aerobic | 10.9 |
| | Anaerobic | 10.4 |

After humification, the stable manure takes first place as an energy source for the free nitrogen-fixing organisms. This difference may be due to the higher content of cellulose material. Here, unlike Hanzawa's results, green-manure humus, as well as the green manure, enhanced nitrogen fixation.

In his study of the carbon and nitrogen balance in the soil, Felber (7) applied dried, pulverized straw and obtained nitrogen fixation in both sand and and clay soils. Brown and Allison (4) treated soil in pots with stable manure, straw, stover, and hay. Both leguminous and non-leguminous hays were employed. A gain in nitrogen was the result of these treatments. The tissue from non-legumes gave a greater gain than that from legumes.

Because of their bearing on the experiments described in this paper, the results of Dvorak (6) deserve special consideration. He found that varying amounts of nitrogen are fixed when *Azotobacter* is grown in a solution containing carbohydrates from different sources. Comparisons were made of the amount of nitrogen fixed in the presence of glucose with that fixed from

oak leaves, clover, maple leaves, straw, alfalfa, corn stalks, lupine, corn roots and stubble, and pine needles. These materials were dried, ground, and 10-gram portions added to 250 cc. of tap water containing 1 per cent of calcium carbonate and 1 per cent of dibasic potassium phosphate, except clover, lupine, and alfalfa which were added in a green, crushed form. This solution was placed in 2-liter Erlenmeyer flasks, sterilized, and inoculated with a pure culture of *Azotobacter chroococcum*. The gain in nitrogen is shown in the following table adapted from Dvorak's results.

| MATERIAL | NITROGEN ASSIMILATED IN 100 CC. OF SOLUTION AFTER FOUR WEEKS |
|---|---|
| | *mgm.* |
| Glucose | 23.04 |
| Corn roots and stubble | 7.36 |
| Clover | 7.12 |
| Straw (wheat) | 5.60 |
| Corn stalks | 4.48 |
| Oak leaves | 2.24 |
| Lupine | 2.20 |
| Alfalfa | 1.72 |
| Maple leaves | 1.48 |
| Pine needles | 0.88 |

The value of corn stalks for nitrogen fixation is clearly shown by the figures in the table. Of all the substances, with the exception of glucose, corn stalks gave the highest gain in nitrogen. In a descending scale, clover and wheat straw rank next in stimulating nitrogen fixation. The indirect beneficial effect of plant tissue on nitrogen fixation is clearly established from the results given in this table. Dvorak concluded that the greatest gain in atmospheric nitrogen by *Azotobacter chroococcum* was obtained from compounds with low carbon and with high oxygen content. He thought the small fixation of nitrogen in the case of pine needles was due partly to the presence of terpenes and tannic acid.

Stoklasa (24) tested the availability of various sugars for *Azotobacter chroococcum*. A comparison of arabinose, saccharose, xylose, glucose, galactose, fructose, rhamnose, lactose, and maltose showed that in liquid cultures the highest fixation of nitrogen occurred with arabinose and the lowest with rhamnose.

When *Azotobacter* was grown on polysaccharides, various alcohols, sugars, aliphatic and non-aliphatic organic acids, substances occuring widely in nature Mockeridge (21) noted fixation of nitrogen by this organism.

From the preceding data, it seems certain that in a soil with the proper reaction, the common soil flora should give an increase in nitrogen provided a suitable source of energy is added. Based on these results, a series of experiments was planned to study the effect of green manures on nitrogen fixation.

## METHODS

Two common field crops, a legume and a non-legume, were selected. These plants were chosen because of their difference in content of nitrogen and pentosan. The results of many analyses by Düring (5) show that non-legumes are, as a rule, much higher in pentosans than are legumes. Since pentosans are easily hydrolized to pentose sugars, they are important from the standpoint of the independent nitrogen-fixing organisms.

Red clover (*Trifolium pratense*), oats (*Avena sativa*), and wheat (*Triticum vulgare*) were used as green manures. The plants were cut into fine pieces with scissors and 1.5 per cent of the green tissue added to the soil, or 0.5 per cent on the dry basis. This amount was selected because it approximates a good crop of wheat, oats, or clover. Total nitrogen analyses were made of five representative samples of tissue at the time it was added to the soil. The average of these analyses is given in the tabular data under the heading "nitrogen added to 100 grams of soil." The nitrogen of the green manure was subtracted from the total analyses of the treated soil.

Two types of soil were employed, one a field soil classed as Miami silt loam, and the other a garden soil classed as a member of the Clyde series. These two soils were selected because of their difference in organic matter. The field soil is medium low, while the garden soil is high in organic substances. The soils were sieved thoroughly, mixed, and 400-gram portions on the dry basis were weighed out. The green tissue was then added to the soil and the whole thoroughly mixed. In order to secure aeration, the soil was placed in shallow soup plates. These were incubated at 25°C. The soil moisture was maintained at 25 per cent, which represented about half saturation. At regular intervals, the loss by evaporation was replaced by the addition of distilled water.

The first total nitrogen determination was made after 2 weeks and again after 4, 6, and 8 weeks. For each analysis one plate of soil from each similarly treated group was used. The soil was dried, pulverized, and thoroughly mixed on glazed paper. Ten-gram samples were then taken for analysis.

In all nitrogen determinations the greatest care was exercised. Only the purest chemicals were used. The contents of the flasks were digested until clear and the heating continued for another hour to insure complete digestion. All analyses, unless otherwise stated, were made according to the Kjeldahl method modified to include nitrate nitrogen. The reagents were made up in amounts large enough to last throughout the work, thus insuring greater uniformity in results. The standard acid and alkali were frequently checked from time to time. The period of distillation lasted for at least an hour. The standard acid and alkali in these experiments were of N/10 normality. Cochineal was used as an indicator.

*Nitrogen-fixation in garden soil treated with green clover and green wheat*

A series of plates was arranged as follows:

a. Four plates of garden soil untreated.

b. Four plates of garden soil plus 1.5 per cent of green clover.

c. Four plates of garden soil plus 1.5 per cent of green wheat.

The results of this experiment are recorded in table 1.

TABLE 1

*Effect of green clover and green wheat on nitrogen fixation in garden soil*

| NO. | TREATMENT | NITROGEN ADDED TO 100 GRAMS OF SOIL | NITROGEN IN 100 GM. OF SOIL | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | After two weeks | | | After four weeks | | | After six weeks | | | After eight weeks | | |
| | | | Total | Average | Increase | Total | Average | Increase | Total | Average | Increase | Total | Average | Increase |
| | *per cent* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| 1 | None | None | 413.0 | | | 410.2 | | | 410.2 | | | 406.0 | | |
| 2 | None | None | 417.2 | 416.4 | | 410.2 | 409.8 | | 411.6 | 409.4 | | 407.4 | 406.0 | |
| 3 | None | None | 417.2 | | | | | | 407.4 | | | 406.0 | | |
| 4 | None | None | 417.2 | | | 411.6 | | | 410.2 | | | 403.2 | | |
| 5 | None | None | 417.2 | | | 407.4 | | | 407.4 | | | 407.4 | | |
| 6 | 1.5 clover | 13.2 | 423.6* | | | 413.8 | | | 413.8 | | | 406.8 | | |
| 7 | 1.5 clover | 13.2 | 419.4 | 421.4 | 5.0 | 412.4 | 412.7 | 2.9 | 413.8 | 412.7 | 3.3 | 405.4 | 407.9 | 1.9 |
| 8 | 1.5 clover | 13.2 | 422.2 | | | 411.0 | | | 412.4 | | | 408.2 | | |
| 9 | 1.5 clover | 13.2 | 418.0 | | | 413.8 | | | 412.4 | | | 408.2 | | |
| 10 | 1.5 clover | 13.2 | 423.6 | | | 412.4 | | | 411.0 | | | 411.0 | | |
| 11 | 1.5 wheat | 11.5 | 425.3 | | | 412.7 | | | 415.5 | | | 416.9 | | |
| 12 | 1.5 wheat | 11.5 | 426.7 | 422.2 | 5.8 | | 412.3 | 2.5 | 415.5 | 413.8 | 4.4 | 411.3 | 411.6 | 5.6 |
| 13 | 1.5 wheat | 11.5 | 418.3 | | | 409.9 | | | 414.1 | | | 411.3 | | |
| 14 | 1.5 wheat | 11.5 | 416.9 | | | 414.1 | | | 411.3 | | | 414.1 | | |
| 15 | 1.5 wheat | 11.5 | 423.9 | | | 412.7 | | | 412.7 | | | 404.3 | | |

* The green manure nitrogen was subtracted from the total nitrogen of the treated soil.

Different plates of soil were used for each analysis instead of taking samples of soil from the same plate. The decrease in nitrogen of the untreated soil after four, six, and eight weeks is due most probably to a change in methods and not to any actual loss of nitrogen. All the figures given in the vertical columns represent the results of analyses made at the same time, i.e., the methods followed were alike.

It will be seen by an examination of the vertical columns of table 1 and figure 1 that in every case the treated soil, after allowance is made for the green-manure nitrogen present, showed a gain in nitrogen over the untreated. There was some variation in the amount of nitrogen assimilated depending

upon the period of incubation. The greatest gain was obtained two weeks after the green manure was added.

A comparison of these two green manures indicates that clover is more beneficial during the early stages of decomposition, while wheat is beneficial for a much longer period of time. Apparently the non-legume gives a greater gain in nitrogen than the legume. This difference in favor of wheat is perhaps due to the higher content of carbohydrate material.

Under the conditions of this experiment, it seems safe to conclude that green manures as clover and wheat when added to soil furnish some substance which benefits the nitrogen-fixing flora.

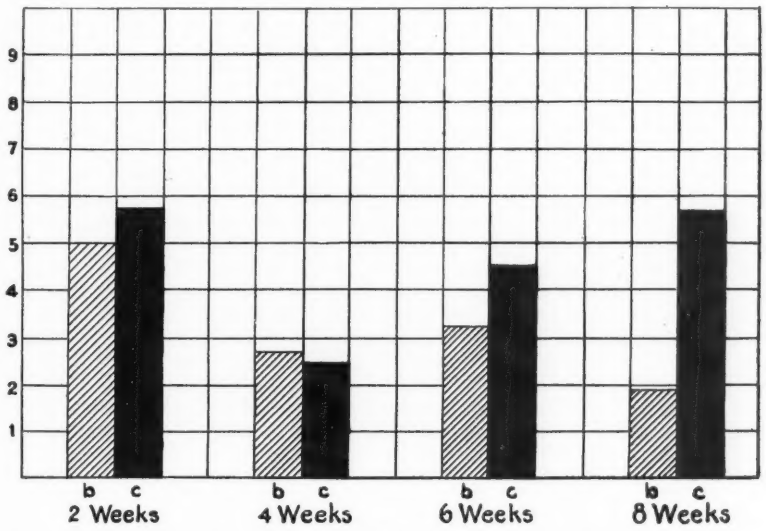



FIG. 1. MILLIGRAMS OF NITROGEN FIXED IN 100 GRAMS OF GARDEN SOIL AS A RESULT OF ADDING GREEN MANURE

*b*, clover-treated soil; *c* wheat-treated soil.

*Nitrogen fixation in field soil treated with green clover and with green oats*

In order to measure the effect of green manures on nitrogen fixation in field soil, a series of plates was arranged similar to those in the preceding test, with the exceptions that green wheat was replaced by green oats and a different sample of green clover was used. The results of previous study showed that the field soil was lower than the garden soil in both organic matter and the total number of bacteria. For these reasons it seemed desirable to make a study of the effect of green manuring on nitrogen fixation in field soil. The arrangement of the experiment and the results secured are shown in table 2.

From the data in table 2 and figure 2, it will be seen that in every case with field soil the treated soil showed a gain in nitrogen beyond that of the untreated. The results, in many instances, are similar to those of the preceding test. The greatest gain of nitrogen was shown two weeks after the green manures were added. The amount of nitrogen fixed varied at the different incubation periods.

Here, as in table 1, a comparison of these two green manures indicates that clover is more beneficial during the early stages of decomposition, while oats are beneficial for a much longer period of time. From the data obtained with

TABLE 2

*Effect of green clover and green oats on nitrogen fixation in field soil*

| NO. | TREATMENT | NITROGEN ADDED TO 100 GMS. OF SOIL | NITROGEN IN 100 GRAMS OF SOIL | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | After two weeks | | | After four weeks | | | After six weeks | | | After eight weeks | | |
| | | | Total | Average | Increase | Total | Average | Increase | Total | Average | Increase | Total | Average | Increase |
| | *per cent* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| 1 | None | None | 274.4 | | | 273.0 | | | 277.2 | | | 284.2 | | |
| 2 | None | None | 274.4 | | | 274.4 | | | 280.0 | | | 285.6 | | |
| 3 | None | None | 271.6 | 272.4 | | 275.8 | 275.2 | | 277.2 | 279.7 | | 285.6 | 285.6 | |
| 4 | None | None | 270.2 | | | 277.2 | | | 281.4 | | | 287.0 | | |
| 5 | None | None | 271.6 | | | 275.8 | | | 282.8 | | | 285.6 | | |
| 6 | 1.5 clover | 15.3 | 281.5 | | | 280.1 | | | 284.3 | | | 288.5 | | |
| 7 | 1.5 clover | 15.3 | 281.5 | | | 281.5 | | | 281.5 | | | 288.5 | | |
| 8 | 1.5 clover | 15.3 | 285.7 | 283.2 | 10.8 | 281.5 | 280.1 | 4.9 | 280.1 | 282.1 | 2.4 | 287.1 | 289.1 | 3.5 |
| 9 | 1.5 clover | 15.3 | 281.5 | | | 278.7 | | | 280.1 | | | 289.9 | | |
| 10 | 1.5 clover | 15.3 | 285.7 | | | 278.7 | | | 284.3 | | | 291.3 | | |
| 11 | 1.5 oats | 7.3 | 282.5 | | | 282.5 | | | 282.5 | | | 292.3 | | |
| 12 | 1.5 oats | 7.3 | 283.9 | | | 282.5 | | | 288.1 | | | 292.3 | | |
| 13 | 1.5 oats | 7.3 | 279.7 | 281.9 | 9.5 | 283.9 | 284.2 | 9.0 | 282.5 | 284.5 | 3.8 | 290.9 | 292.2 | 6.6 |
| 14 | 1.5 oats | 7.3 | 283.9 | | | 282.5 | | | 285.3 | | | 292.3 | | |
| 15 | 1.5 oats | 7.3 | 279.7 | | | 289.5 | | | 283.9 | | | 293.1 | | |

field soil, it is apparent that the non-legume gives a greater gain in nitrogen than the legume. This difference is most likely due to a higher content of carbohydrate material in the oats than in the clover.

There is considerable variation in nitrogen in soil receiving the same treatment when examined at different intervals. These samples, as in the preceding experiment, were taken from different plates throughout the test instead of from the same plate. However, in every analysis the treated soil showed a gain in nitrogen beyond that of the untreated when examined at the same time. In comparing the figures of table 1 with those of table 2 the results

indicate that field soil responds to green manuring, i.e., nitrogen fixation, better than the garden soil.

It appears that green manures as clover and oats when added to soil furnish some substance which benefits the nitrogen-fixing flora.

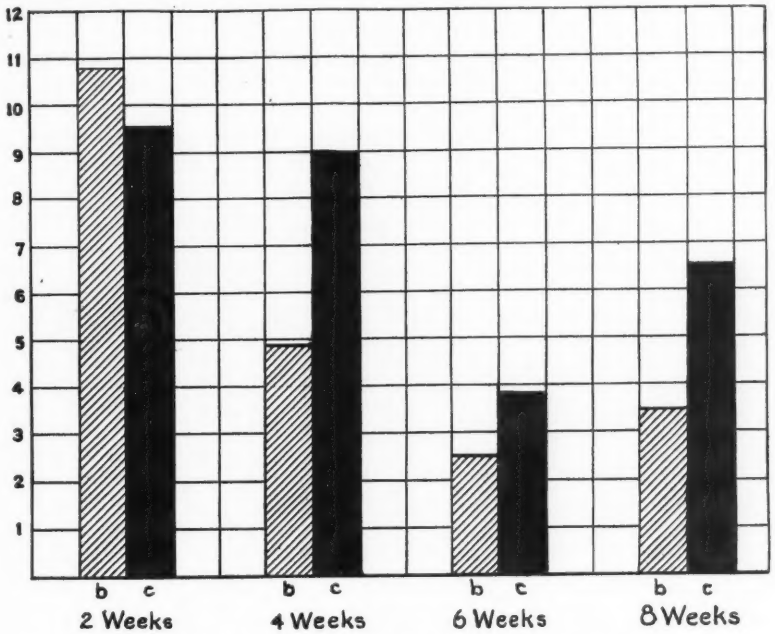



FIG. 2. MILLIGRAMS OF NITROGEN FIXED IN 100 GRAMS OF FIELD SOIL AS A RESULT OF ADDING GREEN MANURE

*b*, clover-treated soil; *c*, oats-treated soil.

*Nitrogen fixation in field soil treated with green clover, green oats, and 0.5 per cent of mannit*

This experiment was made in order to determine whether an increase in nitrogen could be shown with the addition of a green manure in the presence of a small amount of mannit. The treatment was as follows:

a. Two plates of field soil plus 0.5 per cent of mannit.

b. Two plates of field soil plus 0.5 per cent of mannit plus 1.5 per cent of green clover.

c. Two plates of field soil plus 0.5 per cent of mannit plus 1.5 per cent of green oats.

The samples of this series were analyzed after four and eight weeks. The results of this work are presented in table 3.

In reviewing the data in table 3 and figure 3, it will be clearly seen that soil treated with green manure and mannit gave a decided gain in nitrogen beyond that obtained with the mannit alone. Here, as in the preceding experiments, the amount of nitrogen fixed varied with the time of incubation, especially with oats. The greatest gain is noted with oats 4 weeks after treatment. While the results vary somewhat, there is shown in every case a beneficial effect from adding green manures to soil of which the nitrogen-fixing flora had previously received a stimulus by the addition of mannit.

TABLE 3

*Effect of green clover and green oats on nitrogen fixation in field soil containing 0.5 per cent of mannit*

| NO. | TREATMENT | NITROGEN ADDED TO 100 GRAMS OF SOIL | NITROGEN IN 100 GRAMS OF SOIL | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | After four weeks | | | After eight weeks | | |
| | | | Total | Average | Increase | Total | Average | Increase |
| | *per cent* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| 1 | None | None | 282.8 | | | 289.8 | | |
| 2 | None | None | 275.8 | | | 284.2 | | |
| 3 | None | None | 277.2 | 276.9 | | 289.8 | 286.2 | |
| 4 | None | None | 274.4 | | | 282.8 | | |
| 5 | None | None | 274.4 | | | 284.2 | | |
| 6 | 1.5 clover | 15.3 | 277.3 | | | 291.3 | | |
| 7 | 1.5 clover | 15.3 | 278.7 | | | 289.9 | | |
| 8 | 1.5 clover | 15.3 | 281.5 | 279.2 | 2.3 | 291.3 | 290.2 | 4.0 |
| 9 | 1.5 clover | 15.3 | 280.1 | | | 287.1 | | |
| 10 | 1.5 clover | 15.3 | 278.7 | | | 291.3 | | |
| 11 | 1.5 oats | 7.3 | 290.9 | | | 289.5 | | |
| 12 | 1.5 oats | 7.3 | 293.7 | | | 289.5 | | |
| 13 | 1.5 oats | 7.3 | 292.3 | 292.3 | 15.4 | 290.9 | 289.8 | 4.0 |
| 14 | 1.5 oats | 7.3 | 295.1 | | | 290.9 | | |
| 15 | 1.5 oats | 7.3 | 289.5 | | | 288.1 | | |

Therefore, under the conditions of this experiment, it seems safe to conclude that the green clover and the green oats benefited the nitrogen-fixing organisms.

### *Effect of green manures on nitrogen fixation in solution*

If green manures cause an increase in the number of nitrogen-fixing bacteria it seems probable that green-manured soil will give a corresponding gain when used to inoculate Ashby's (1) mannit solution. Aside from the greater number of nitrogen-fixing bacteria, it is very probable that the mannit added in the inoculum will have a beneficial effect on nitrogen fixation.

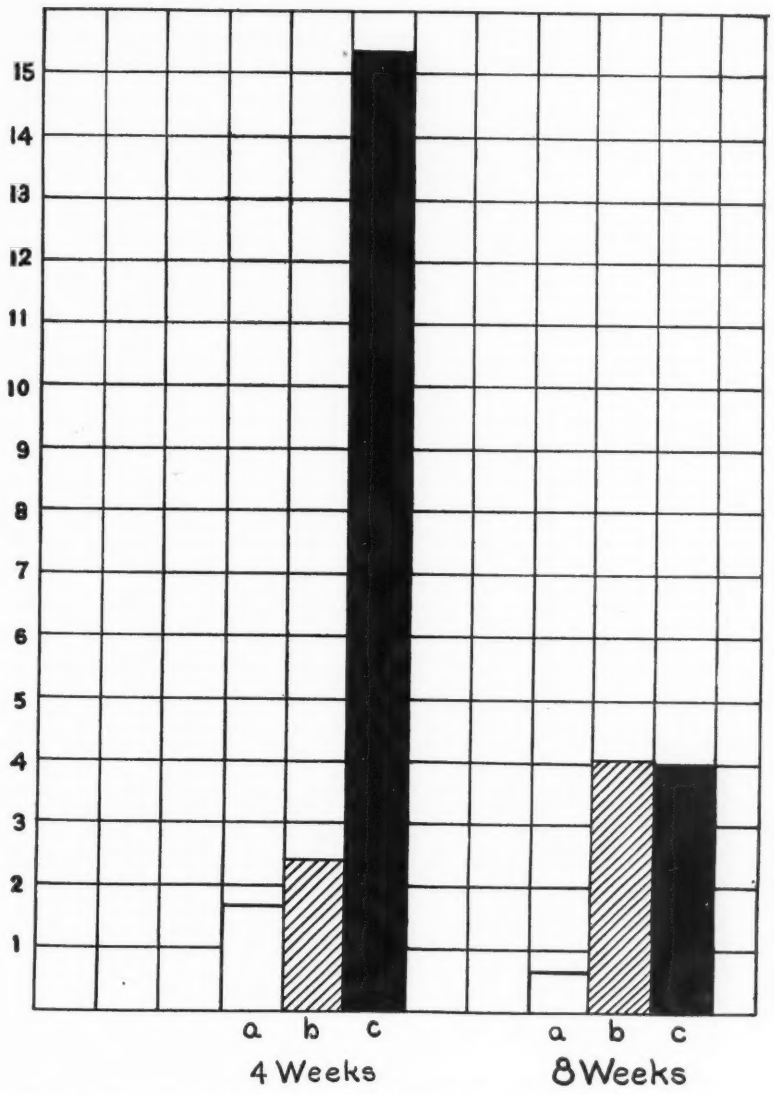



FIG. 3. MILLIGRAMS OF NITROGEN FIXED IN 100 GRAMS OF FIELD SOIL AS A RESULT OF ADDING GREEN MANURE TO SOIL ADDITIONALLY TREATED WITH MANNIT

*a*, soil with 0.5 per cent mannit; *b*, clover-treated soil + 0.5 per cent mannit; *c*, oats-treated soil + 0.5 per cent mannit.

In order to measure the effect of green manuring on the nitrogen gain in solution, 500 cc. Erlenmeyer flasks, each containing 100 cc. of mannit solution, were inoculated with 10 gram portions of soil. Six flasks were inoculated from each plate of soil. One-half of these were immediately sterilized to serve as controls. These inoculations were made at the beginning of the experiment and repeated at intervals of two weeks, i.e., zero, two, four, six, and eight-

TABLE 4

*Effect of green clover and green wheat on nitrogen fixation of garden soil in solution*

| NO. | TREATMENT | GREEN-MANURE NITROGEN ADDED IN 100 CC. OF SOLUTION | NITROGEN IN 100 CC. OF SOLUTION AFTER | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | No weeks Incubated 16 days | | Two weeks Incubated 10 days | | Four weeks Incubated 7 days | | Six weeks Incubated 14 days | | Eight weeks Incubated 14 days | |
| | | | Total | Increase due to green manure | Total | Increase due to green manure | Total | Increase due to green manure | Total | Increase due to green manure | Total | Increase due to green manure |
| | *per cent* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| 1 | None sterile | None | 41.02 | | 41.58 | | 44.38 | | 41.72 | | 42.98 | |
| 2 | None sterile | None | | | 41.44 | | 43.40 | | 42.14 | | 42.88 | |
| 3 | None sterile | None | 41.02 | | 41.72 | | 43.40 | | 42.56 | | 43.40 | |
| 4 | None not sterile | None | 49.42 | | 50.26 | | 53.06 | | 51.80 | | 50.96 | |
| 5 | None not sterile | None | 49.28 | | 50.96 | | 52.92 | | 52.36 | | 50.40 | |
| 6 | None not sterile | None | 49.70 | | 51.52 | | 52.08 | | 50.96 | | 52.08 | |
| 7 | 1.5 clover sterile | 1.32 | 42.08 | | 41.66 | | 42.78 | | 41.94 | | 42.22 | |
| 8 | 1.5 clover sterile | 1.32 | 41.80 | | 42.36 | | 42.92 | | 41.52 | | | |
| 9 | 1.5 clover sterile | 1.32 | 42.64 | | 42.22 | | 42.54 | | 41.94 | | 43.34 | |
| 10 | 1.5 clover not sterile | 1.32 | 51.88 | | 51.88 | | 53.00 | | 52.58 | | 52.86 | |
| 11 | 1.5 clover not sterile | 1.32 | | 1.27 | 51.88 | 0.60 | 52.72 | 0.92 | | 1.88 | 53.56 | 1.73 |
| 12 | 1.5 clover not sterile | 1.32 | 51.88 | | 52.28 | | 52.16 | | 51.88 | | 51.88 | |
| 13 | 1.5 wheat sterile | 1.15 | 41.55 | | 41.13 | | 42.39 | | 41.97 | | 41.83 | |
| 14 | 1.5 wheat sterile | 1.15 | 40.71 | | 41.69 | | | | 41.69 | | 41.83 | |
| 15 | 1.5 wheat sterile | 1.15 | 41.41 | | 41.55 | | 42.39 | | 41.83 | | 41.55 | |
| 16 | 1.5 wheat not sterile | 1.15 | 50.65 | | 55.85 | | 53.31 | | 50.79 | | 50.93 | |
| 17 | 1.5 wheat not sterile | 1.15 | 50.23 | 1.13 | 54.15 | 3.78 | 52.61 | 1.49 | 51.35 | 0.69 | 51.77 | 1.27 |
| 18 | 1.5 wheat not sterile | 1.15 | 51.49 | | 54.71 | | 52.61 | | 51.07 | | 50.93 | |

week periods after the manure was added. All of the cultures were kept at 25°C. The time of incubation varied from seven to sixteen days; for the first set of inoculations, sixteen days; for the second set, ten days; for the third, seven days; and for the fourth and fifth tests, fourteen days. It was observed by Koch and Seydel (17) that *Azotobacter* assimilated the greater

part of its nitrogen in the early stages of growth. In order to determine whether the organisms assimilating nitrogen were stimulated soon after inoculations were made, the cultures were analyzed after short periods of incubation. The maximum length of incubation did not exceed sixteen days.

At the end of each incubation period a total nitrogen determination of each flask with the Kjeldahl method not to include nitrates, was made. The results of the analyses are recorded in table 4.

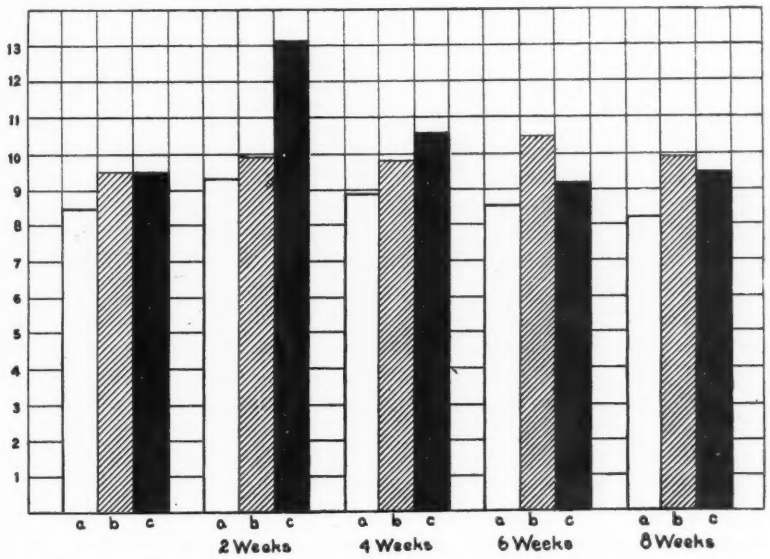



FIG. 4. MILLIGRAMS OF NITROGEN FIXED IN 100 CC. OF ASHBY'S SOLUTION INOCULATED WITH TREATED AND UNTREATED SOIL

Each set was inoculated at intervals of two weeks after starting the experiment, and these allowed to incubate for varying periods. *a*, untreated soil; *b*, clover-treated soil; *c*, wheat-treated soil.

The data in table 4 and figure 4 show that in every case green manures enhanced nitrogen fixation. Although the gain in nitrogen is not large, however, because of a number of parallel determinations, it seems safe to conclude that these substances benefit the fixation of nitrogen in solution. Wheat did not prove to be superior to clover as a stimulus to nitrogen fixation in solution. Clover gave the best results from inoculations made at the beginning of the treatment, and after six and eight week periods. Wheat did better than clover after decomposing for two and four weeks. However, the maximum gain was noted with wheat after two weeks. In general these results fail to show any relation between the age of decomposing manure and the amount of nitrogen assimilated.

This increase in fixation may be due to one or more agencies: first a greater number of nitrogen-fixing bacteria; and second, to the green manure added in the inoculum. Because of the small amount of green manure in the inoculum, it seems that the greater number of bacteria, as a result of the soil treatment, is probably the chief factor concerned with the gain in nitrogen.

*Fixation of nitrogen by pure cultures of bacteria*

For this test green wheat (*Triticum vulgare*) and red clover (*Trifolium pratense*) were crushed into a semi-fluid mass by grinding for several hours in a porcelain ball mill. To 1 liter of Ashby's solution, minus the mannit, 50 grams of the crushed tissue were added and the reaction adjusted to the neutral point of phenolphthalein. One hundred cubic-centimeter portions of the mixture were measured and poured into 1-liter Erlenmeyer flasks. Each 100 cc. portion represented 5 grams of green tissue or 1 gram of dry material. Both the oats and clover were near maturity and contained smaller amounts of moisture than the preceding samples. The flasks and contents were then sterilized in the autoclave and inoculated with a pure culture of *Azotobacter chroococcum* which had been grown on a medium of the same formula. This same culture of *Azotobacter* had in previous tests shown a gain of 7.4 mgm. of nitrogen when grown in a 1 per cent mannit medium. One-half of the culture flasks were sterilized immediately after inoculation. These were used as controls. The entire set of flasks was placed in the 26°C. incubator for 28 days. At the end of this period total nitrogen determinations were made. The results are presented in table 5.

From the results presented in table 5, it appears that *Azotobacter*, in using the carbon of green manures, can fix a very small amount of nitrogen. In general the results agree with those of Dvorak (6). The very high amount of nitrogen assimilated in his experiment with green clover was never obtained in these experiments. This may be due in part to a difference in the strain of *Azotobacter* or to a difference in the composition of green tissue. However, it seems safe to assume that the carbohydrate in the green tissue available for this organism is small. Oat tissue apparently furnishes a better carbon source than clover.

Before proceeding to a summary of the results of these experiments, it is desirable to present a brief estimate of the possible value of green manures for nitrogen fixation under field conditions.

If an acre of soil 6 inches deep weighs 2,000,000 pounds, then the turning under of 30,000 pounds of green manure, or 1.5 per cent by weight of soil, should be followed by a nitrogen increase of 3 to 5 mgm. of nitrogen in 100 grams of soil, or 60 to 100 pounds of nitrogen in one acre of soil.

TABLE 5

*Nitrogen assimilated by Azotobacter chroococcum in solution containing green clover and green oats*

Incubated for 28 days at 26°C.

| NO. | TREATMENT | NITROGEN IN 100 CC. OF SOLUTION | | | | | |
|---|---|---|---|---|---|---|---|
| | | Clover | | | Oats | | |
| | | Total | Average | Increase | Total | Average | Increase |
| | | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| 1 | Sterile | 23.5 | | | 16.8 | | |
| 2 | Sterile | 23.7 | | | 16.8 | | |
| 3 | Sterile | 23.7 | | | 16.8 | | |
| 4 | Sterile | 23.7 | 23.7 | | 16.8 | 16.8 | |
| 5 | Sterile | 23.7 | | | 16.7 | | |
| 6 | Sterile | 23.5 | | | 16.9 | | |
| 7 | Sterile | 23.9 | | | 16.7 | | |
| 8 | Sterile | 23.9 | | | 16.7 | | |
| 9 | Azotobacter | 24.6 | | | 17.8 | | |
| 10 | Azotobacter | 24.4 | | | 17.9 | | |
| 11 | Azotobacter | 24.3 | | | 17.6 | | |
| 12 | Azotobacter | 24.3 | 24.4 | 0.7 | 17.6 | 17.7 | 0.9 |
| 13 | Azotobacter | 24.4 | | | 17.6 | | |
| 14 | Azotobacter | 24.4 | | | 17.6 | | |
| 15 | Azotobacter | 24.4 | | | 17.8 | | |
| 16 | Azotobacter | 24.2 | | | 17.6 | | |

## SUMMARY

The results of all experiments show an increase in the assimilation of nitrogen due to the applications of green clover, wheat, or oats. This increase is noted both in solution and soil tests, and with pure cultures of *Azotobacter* growing in a medium containing green tissue.

Two kinds of soil were used in these experiments and in each case a small gain in nitrogen was observed. The variation in nitrogen content, in the untreated soil, is no doubt due to the error of analysis. The contents of separate plates were taken for analysis instead of portions of soil from the same container.

The green manures, probably because of the nature of their carbon content, stimulated nitrogen fixation very soon after treatment. It is interesting to note that in the majority of cases, the greatest gain resulted with the use of non-legume tissue as wheat and oats. These materials contained less nitrogen and more carbohydrate material, and decomposed more slowly than clover.

## CONCLUSIONS[3]

1. From the data obtained under the conditions of the preceding experiments, it seems safe to conclude that green manures, as clover, wheat, or oats, when added to soil favor free nitrogen fixation.

2. A gain in nitrogen was noted in both soils. The increase was highest with field soil, due perhaps to a greater number of nitrogen-fixing organisms.

3. A gain in nitrogen was observed (a) where green tissue was applied to soil previously treated with a small amount of mannit; (b) where treated soil was used to inoculate Ashby's solution; and (c) when a pure culture of *Azotobacter* was used to inoculate a sterile solution containing green tissue.

4. The non-legume tissue stimulated fixation more than the legume, probably because of the nature of its carbohydrate content.

5. The results of these experiments in their entirety show that nitrogen fixation results from the addition of green manures to soil. Fortunately for the agriculturist, the plants giving best results are the ones lowest in nitrogen content.

## REFERENCES

(1) ASHBY, S. F. 1907 Some observations on the assimilation of atmospheric nitrogen by a free-living soil organism—*Azotobacter chroococcum* of Beijerinck. *In* Jour. Agr. Sci., v. 2, p. 38.

(2) BERTHELOT, M. 1885 Fixation directe de l'azote atmosphérique libre par certains terrains argileux. *In* Compt. Rend. Acad. Sci. [Paris], t. 101, p. 775–784.

(3) BOTTOMLEY, W. B. 1913 Effects of soluble humates on nitrogen fixation and plant growth. *In* Rpt. Brit. Assoc. Adv. Sci., 1913, p. 771.

(4) BROWN, P. E., AND ALLISON, F. E. 1916 The influence of some common humus-forming materials of narrow and of wide nitrogen-carbon ratio on bacterial activities. *In* Soil Sci., v. 1, no. 1, p. 49–75.

(5) DÜRING, F. 1897 Über den Pentosangehalt verschiedener Futtermittel und deren Rohfaser. *In* Jour. Landw., Bd. 45, p. 79–95.

(6) DVORAK, J. 1912 Studien über die Stickstoffanhäufung im Boden durch Mikroorganismen. *In* Ztschr. Landw. Versuchs. Oesterr., Bd. 15, p. 1077–1097.

(7) FELBER, P. 1915 Über das Gleichgewicht zwischen Stickstoff und Kohlenstoff im Boden. *In* Mitt. Landw. Lehrk. K. K. Hochschule Bodenk, Wien, Bd. 3, Heft 1, p. 23–54.

(8) HANZAWA, J. 1914 Einige Beobachtungen über Stickstoff-Bindung durch Azotobacter in Stickstoffarmen und Stickstoffreichen Substraten. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 41, p. 573–576.

(9) HENRY, E. 1902 Fixation de l'azote atmosphérique par les feuilles mortes en forêt. *In* Ann. Sci. Agron., t. 1–11, p. 313–317.

(10) HOFFMANN, C. 1913 Increase in nitrogen fixation of soil due to applications of carbohydrates. Wis. Agr. Exp. Sta. Bul. 288, p. 27.

(11) HORNBERGER, H. 1906 Nochmals Streu und Stickstoff. *In* Ztschr. Forst. u. Jagdw., Bd. 38, pp. 775–781. *Abs. in* Exp. Sta. Rec., v. 18, p. 643.

(12) HUTCHINSON, H. B. 1912 Studies on nitrogen assimilation by the free living organisms. *In* Rpt. Brit. Assoc. Adv. Sci., 1912, p. 740–741.

---

[3] The writer is indebted to Dr. E. B. Fred for suggestions and supervision of both the work and preparation of this paper.

(13) KOCH, A. 1909 Weitere Untersuchungen über die Stickstoffanreicherung des Bodens durch freilebende Bakterien. *In* Jour. Landw., Bd. 57, p. 269–286.

(14) KOCH, A. 1910 Über Luftstickstoffbindung im Boden mit Hilfe von Zellulose des Energie Material. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 27, p. 1–7.

(15) KOCH, A. 1913. Ergebnisse Zehnjähriger vergleichender Feldversuche über die Wirkung von Brache, Stalldünger und Klee. *In* Jour. Landw., Bd. 61, p. 263.

(16) KOCH, A., et al. 1907 Die Stickstoffanreicherung des Bodens durch freilebende Bakterien und ihre Bedeutung für die Pflanzennährung. *In* Jour. Landw., Bd. 55, p. 355–416.

(17) KOCH, A., AND SEYDEL, S. 1911 Versuch über den Verlauf Stickstoffbindung durch Azotobacter. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 31, p. 570–577.

(18) KRZEMIENIEWSKI, S. 1909 Untersuchungen über *Azotobacter Chroococcum*. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 23, p. 161–173.

(19) LÖHNIS, F., AND GREEN, H. H. 1914 Über die Entstehung und die Zersetzung von Humus, sowie über dessen Einwirkung auf die Stickstoffassimilation. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 40, p. 52–60.

(20) MARR, F. S. 1910 Denitrifikation und Stickstoffsammlung im Ackerboden. *In* Mitt. Landw. Inst. Breslau, Bd. 5, p. 639–656.

(21) MOCKERIDGE, FLORENCE A. 1915 Some organic matter as culture medium for Azotobacter. *In* Biochem. Jour., v. 9, no. 2, p. 272–283.

(22) PRINGSHEIM, H. 1910 Weiteres über die Verwendung von Cellulose als Energiequelle zur Assimilation des Luftstickstoffs. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 26, p. 222–227.

(23) REMY, T., AND RÖSING, G. 1911 Über die biologische Reizwirkung naturlicher Humusstoffes. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 30, p. 349–384.

(24) STOKLASA, J. 1908 Beitrag zur Kenntnis der chemischen Vorgänge bei der Assimilation des elementaren Stickstoffs durch Azotobacter und Radiobacter. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 21, p. 484–509.

(25) SÜCHTING, M. 1905 Die Assimilation des freien Stickstoffs im toten Laub der Waldbaüme. *In* Hanov. Land. u. Forstw. Ztg., Bd. 58; *Abs. in* Centbl. Bakt. [etc.], Abt. 2, Bd. 14, p. 342.

# THE INFLUENCE OF FINENESS OF DIVISION OF PULVERIZED LIMESTONE ON CROP YIELD AS WELL AS THE CHEMICAL AND BACTERIOLOGICAL FACTORS IN SOIL FERTILITY[1]

NICHOLAS KOPELOFF

*Research Fellow, Rutgers College*

Received for publication April 30, 1917

## REVIEW OF LITERATURE

If the value of an agricultural practice is determined by its age and universality, then that of liming must be well established, for the use of lime has been common to all lands since antiquity. Sound instinct has determined the use of lime and it is only the recent vogue of commercial fertilizers that has prevented liming from receiving the consideration which is its due. It is well known that the choice of one form of lime over another depends of necessity upon a variety of factors, such as soil texture, of organic matter content, items of cost, etc. It has been generally assumed in the past that burnt or slaked lime is more efficient than ground limestone for agricultural purposes. While much evidence has been offered to substantiate this claim, nevertheless, recent investigations have paved the way for the belief that limestone ground sufficiently fine may be considered as equivalent, if not superior, in value to the caustic forms of lime. Consequently, it is desirable to establish the possibilities and limitations of such contentions. To this end, one promising field of endeavor has furnished the basis for this investigation, namely, how do different grades of pulverized limestone influence crop yields, the chemical and bacteriological factors in soil fertility, and furthermore, how do they compare with the effects produced by burnt lime? More particularly the problem has been attacked from the following standpoints: the effect of different degrees of fineness of pulverized limestone on crop yields on different soils; the rate of neutralization of soil acidity; the bacteriological processes of ammonification, nitrification and nitrogen-fixation; the loss of limestone in drainage and the composition of the drainage water. While these may at first appear to be primarily scientific considerations, an attempt will be made to indicate their direct practical significance. The conclusions arrived at when taken in conjunction with other considerations may well furnish the basis for choice in the purchase of lime and therefore have an essential bearing upon the farmer's agricultural practice.

In approaching the problem concerning the influence of the different degrees of fineness of division of pulverized limestone, it is of interest to review the investigations pertinent to the subject at hand.

[1] A thesis presented to Rutgers College in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1917.

Heinrich (39) appears to be the first investigator to report such data. The following results were recorded by him in 1908.

| GRADES | PEAS | LUCERNE |
|---|---|---|
| *mm.* | *grams* | *grams* |
| 1.5–2.0 | 32.0 | 17.1 |
| 1.0–1.5 | 38.8 | 22.7 |
| 0.5–1.0 | 43.3 | 28.9 |
| 0.5 | 48.0 | 28.9 |
| No Ca + Fertilizer | 22.6 | 0 |
| No Ca + No fertilizer | 26.0 | 0 |

He concludes from his work that the finer the grade of limestone the better the results obtained.

Voelcker (110) has compared "coarsely ground with finely ground" limestone and burnt lime when applied to the ordinary Woburn soil in the usual manner for the growth of wheat. Although the author does not state the degree of pulverization, nevertheless, the yields with the finely ground material are superior to those obtained with coarser, whether calcium or magnesium limestones were employed. Further, burnt lime was inferior to ground limestone whether coarse or fine. This result, Voelcker maintains, "is quite borne out by experience on the field scale in the Stackyard Field (plot 2bb) where applications of burnt lime have not shown good results at the outset in the case of wheat, but have required time to "work." This opens up the question as to whether the "alkalinity" of the burnt lime applied has had an influence in lowering the crop yield; also a further question as to whether the changes due to the use of lime may not be of a biological rather than a chemical nature." This interesting line of work is being prosecuted further by the author.

H. von Feilitzen (22) has compared slaked lime with pulverized limestone of the following grades: 0.2 mm., 0.2 to 0.5 mm., 0.5 to 1 mm., 1 to 2 mm. and 2 mm. He finds that slaked lime gives greater yields than calcium carbonate and both increase with an increase in fineness of division. The data, however, are not regarded as conclusive and the need for further experimentation is suggested.

Brehmer (8) treated a loam soil with limestone of different degrees of fineness (no mention being made of the degree of pulverization). The finer the material the greater was the increase in crop yield, while a mixture of 67 per cent $CaCO_3$ with 33 per cent CaO proved superior to the most highly pulverized limestone. In several instances in these experiments the increases between treatments were not greater than between duplicates receiving the same treatment.

Gerlach (35) supports Baessler's (4) contention that the solubility of calcium carbonate in marl increases proportionally with the amount of carbonate it

contains and the increased pulverization of the particles. The matter will bear further discussion in another connection.

The Rhode Island Agricultural Experiment Station (92) has published the early results of an experiment dealing with the growth of mangels and carrots when treated with limestone of varying degrees of pulverization. Their results are as follows:

| TREATMENT | MANGELS, 1913 | CARROTS, 1914 |
|---|---|---|
| | *pounds* | *pounds* |
| No lime | 107 | 175 |
| 10–20 Mesh limestone | 138 | 314 |
| 20–24 Mesh limestone | 204 | 469 |
| 40–80 Mesh limestone | 389 | 563 |
| Unsifted | 279 | 470 |

The material coarser than 1/20 inch had little effect in the first year but considerable effect in the second. Whether this is due to a crop difference or one depending primarily upon increased availability of the limestone is considered questionable. It is evident, however, that in both cases an increase in fineness of division of pulverized limestone was paralleled by an increase in crop yield.

Thomas and Frear (104) have very recently published data indicating the importance of fineness of division in the use of pulverized limestone. The grades of limestone employed by them were as follows: 1/20 to 1/40 inch, 1/40 to 1/60 inch, 1/60 to 1/80 inch, 1/80 to 1/100 inch, and finer than 1/100 inch. Clover was grown in two soils held in paraffined baskets. The air-dry weights recorded indicate a progressive increase in yield as the limestone was more finely divided. This contention is limited by the fact that the authors obtained variations between soils receiving the same treatment as great as the differences between successive treatments. According to Frear the results of this investigation are being corroborated in a continuation series of experiments.

Ames and Schollenberger (3) determined the lasting effects of magnesian as compared with calcareous stone ground to different degrees of fineness. While the crop yields show as yet no marked differences, which is probably due to the high fertility of the soil employed, the authors concluded that the fineness of limestone is an important factor in determining its effectiveness. Material 100 per cent of which passes 10-mesh and 50 per cent of which passes 50-mesh and 35 to 40 per cent of which passes 100-mesh sieves is highly effective as a soil amendment; while material not finer than 1/20 inch produces but slight benefit in correcting soil acidity. Thus the results obtained in Ohio corroborate those of Pennsylvania, where it is recommended that ground limestone should pass at least a 60-mesh sieve.

Since the following experiments included a comparison of burnt lime with

the different grades of pulverized limestone in many instances, it it relevant at this point to consider briefly the previous work bearing upon this subject. Several of our agricultural experiment stations have given this matter considerable attention and especially noteworthy are the results obtained at Pennsylvania which cover a period of more than thirty years (30). The crushed limestone plats have given consistently higher yields than the plats which received the burnt lime dressing. An interesting point, established as a result of pot experimentation, is that on soils having a lime requirement (Veitch) over 1000 pounds of CaO per acre, ground limestone is manifestly superior to calcium oxide, while the differences on more alkaline soils are slight and more variable.

Patterson (85) also conducted field experiments for more than eleven years with caustic lime, by burning both stone and shells, and the carbonate of lime in ground shells and shell marl. The average crops of maize, wheat and hay were all larger on the plats treated with carbonate of lime.

Adams (1) states as a result of comparative work that no appreciable difference was noted in the benefit derived from an application of the same quantity of calcium oxide, whether in the form of ground limestone or in hydrated lime. In a recent bulletin of the Rhode Island Extension News Service a comparison of 80-mesh limestone with slaked lime shows that practically the same yield was obtained in each case

Thorne (106) also has stated that "in actual practice, the experiments made by the Ohio Experiment Station have shown practically no superiority of one form of lime over another, provided the limestone has been so ground that 80 per cent of it will pass a sieve having 100 meshes to the linear inch, and provided also of course, that the two materials are used on the basis of actual calcium contained.

Saul (95) recommends finely ground limestone as superior to burnt lime, without furnishing experimental evidence to that effect.

Hardt (38) obtained larger yields with marl than with calcium oxide when applied in equivalent amounts to a heath soil under a five-year rotation with legumes every year.

Hoffman (41) gives the results of 79 experiments upon 150 farms in 17 regions of the German Empire. In most cases burnt lime was beneficial. On light soils poor in humus and in dry seasons the carbonate was superior to the quick lime.

Meyer (73) grew mustard and beans alternately for four years in pots of sandy loam treated with different forms of lime. As a result of this experiment, the yields resulting from the application of ground limestone and calcium carbonate (c.p.) were calculated as 100 and the burnt lime received then a valuation of only 95. The ground limestone had the following mechanical analysis: 74.3 per cent of flour (equivalent to no. 100 Thomas slag sieve), 21.4 per cent finer than 0.5 mm., and 4.3 per cent larger than 0.5 mm. but smaller than 10 mm.

Mooers (76) reports comparative experiments with ground limestone and caustic lime conducted for four years with a variety of crops including corn, wheat, oats, barley, sorghum, clover, grass, cowpeas and cotton. He concludes that "in these trials two tons of ground limestone proved somewhat superior to one ton of burnt lime."

Voorhees, Lipman and Brown (113) concluded, with certain reservations, from their investigations of the comparative value of different forms of lime that "the carbonate led to the production of more dry matter, more nitrogen and more ash than did the burnt lime."

Porter and Gaut (91) conclude from their comparison of cob (kiln) lime, ground lime and ground limestone as top-dressings for various pastures, with and without manure, that ground limestone was more profitable for grass lands in need of lime than CaO.

Yokayama (124) maintains as a result of experiments with oats grown in sand cultures that precipitated calcium carbonate is far more effective than fine limestone meal in increasing crop yield.

Ellett (19) emphasizes the importance of relative cost in calculations concerning the use of different forms of lime and maintains that ground limestone gives results as good as those obtained with burnt lime.

H. von Feilitzen (21) found that slaked lime gave greater yields than carbonate, but the data are not conclusive. In a later publication (22) the same author reported results obtained showing that calcium carbonate was as valuable as burnt lime on acid soils poor in lime.

Wright (123) in investigations conducted on medium and light soils found that in the beginning burnt lime was superior to gas lime, but that in the seven succeeding years of the rotation, the latter was much more beneficial. In that period it gave a total increase of more than three times the value of that produced by burnt lime and because of the lower cost proved much more profitable.

Riggl (93) found that calcium carbonate as a top-dressing was preferable to burnt lime except where the latter was used in very small amounts. He is of the opinion that burnt lime is better than calcium carbonate because of the physical and bacteriological effects resulting from the finer state of division of the particles.

Voelcker (111) states as a result of some grass experiments that "it is still early for the application to tell, but at the present time if any plots were selected for choice they would be plots 4 and 5 where ground limestone and ground chalk, respectively, have been used. Ground lime also seems to have done better than lump lime."

Barker and Collison (5) discuss the superiority of ground limestone over burnt lime, and, without publishing data, conclude that 10-mesh limestone is to be recommended.

Rothert (94) in noting the depressing effect upon crop yield of increased quantities of burnt lime find that on a loam soil barley gave a greater yield

with 0.5 per cent of calcium carbonate than with 0.25 per cent of burnt lime. In sand the reverse was true.

Lauder, Fagan and Steele (58) record the beneficial effects of ground lime and waste carbonate for finger and toe disease. They state that when the latter product is available it may be employed instead of burnt lime with satisfactory results.

Grantham (36) in a preliminary report asserts that "results thus far indicate that the carbonate of lime is practically as effective as the burned forms of lime in promoting the decomposition of organic matter and the availability of nitrogen."

The effect upon soil reaction of applications of limestone has been the subject of investigation by the Pennsylvania Agricultural Experiment Station (29, 117, 118). In this connection it may be stated that Dr. W. Frear has contributed one of the most valuable and comprehensive surveys of the literature dealing with liming (30). Because of the thoroughness of his recent bulletin it has been unnecessary in the present instance to do more than briefly indicate the nature of the problems attacked by various investigators concerning pulverized limestone. A more complete discussion will be found in the above-mentioned bulletin which has furnished material for this bibliography. In an experiment on nitrification, White (118) found that at the end of four weeks an increase in fineness of division of limestone used was responsible for a corresponding decrease in the lime-requirement of the soil. The influence of crops upon soil reaction has also been an interesting point established by Gardner and Brown (34) in plot experiments with white clover. In general it was found that the soils of the unlimed plots showed an increase in acidity on the average after the crop roots had been removed, and even those limed or treated with fine carbonate of lime in quantity sufficient to neutralize the original acidity were found to be acid to a slight degree after the crop was removed.

Veitch (109) has observed that different plants affect the soil reaction differently.

Lipman and Blair (64) found in the course of field experiments that after a number of years plots (including one series unlimed, the other treated with limestone) were acid; those of the unlimed groups requiring from 1000 to 2000 pounds (average 1840 pounds) of lime per acre, while those of the limestone-treated group averaged about 700 pounds. Volunteer clover sprang up on most of these plots, but its yield on the limed plots was double that on the unlimed.

The beneficial effect of ground limestone upon the growth of legumes has been observed by the following investigators: Bäessler (4), Hardt (38), Lipman (63), Stevenson and Weston (100), Peter and Jones (88) and many others.

Various technical phases as well as general discussions pertaining to ground limestone have been treated in an interesting manner by Ames (2), Bernard

(6), P. E. Brown (14), Dumont (18), Fippin (23), Fischer (25), Frear (30), Frear and Thomas (31), Johnston (50), Morse (77), Neuffer (81), Pfeiffer (90), Simmermacher (99), Strebut (101), Takeuchi (103), Wheeler (115) and Whitson and Weir (119).

## THE EFFECT OF FINENESS OF DIVISION OF PULVERIZED LIMESTONE ON CROP YIELD, NITROGEN CONTENT AND LIME-REQUIREMENT OF VARIOUS SOILS

It is apparent from the foregoing review that there is a paucity of experimental evidence concerning the different grades of pulverized limestone and their relative value compared with the burnt forms of lime. The practical significance which attaches to this problem is responsible for the following greenhouse experiments which were begun in the summer of 1914. Crimson clover was grown on soils which may be regarded as typical of rather extensive fertile areas in the United States. These were respectively designated as Carrington (Iowa) silt loam, Wooster (Ohio) silt loam, Cumberland (Tennessee) silt loam, Norfolk (Virginia) sandy loam, Sierra (California) sandy loam and Portsmouth (New Jersey) acid muck. They were obtained through the courtesy of Dr. P. E. Brown, Prof. C. E. Thorne, Prof. C. A. Mooers, Dr. J. W. Schoene and Dr. J. G. Lipman. The procedure was to fill 10-pound glazed earthenware pots with soil to which was added 2 grams of acid phosphate (14 per cent $P_2O_5$) and 1 gram of potassium chloride (50 per cent $K_2O$). (All soils used in this and following experiments were from unlimed and unfertilized plots and were air-dried, thoroughly mixed, and sieved through a 12-mesh sieve.) Limestone was then added which had been pulverized and hand-sieved as follows: 20 to 40-mesh, 60 to 80-mesh, 100 to 200-mesh and finer than 200-mesh, as well as calcium oxide (c.p.) which had been ground in a mortar. The limestone used was a uniform crystalline grey limestone having the following composition:

| | *per cent* |
|---|---|
| $SiO_2$ | 3.75 |
| $R_2O_3$ | 1.72 |
| $CaCO_3$ | 92.00 |
| $MgCO_3$ | 3.45 |

The lime was added in quantities sufficient to neutralize the soil acidity as determined by the Veitch method. The lime-requirements of the different soils in pounds of CaO per acre were as follows: Carrington silt loam, 3700; Wooster silt loam, 4000; Cumberland silt loam, 3400; Norfolk sandy loam, 2300; Portsmouth acid muck, 8733; and Sierra sandy loam, alkaline. Thirty tested seeds of crimson clover were planted in each pot on February 10. The plants were thinned to 15 in number by April 15 and the pots kept at optimum temperature and moisture conditions, being made up to weight twice weekly. The crop was harvested June 29. During the warm weather thrips attacked the plants and frequent spraying with nicotine sulfate became neces-

sary. However, the crops were not unduly injured. The results of the crop yield (dry matter), per cent of nitrogen, nitrogen content in grams, and original and final lime-requirements are recorded in tables 1 and 2.

TABLE 1

*The effect of fineness of division of pulverized limestone on crimson clover and lime-requirement*

| TREATMENT | WEIGHT OF CROP | DUPLICATE | AVERAGE | NITROGEN | NITROGEN | AVERAGE NITROGEN | TOTAL NITROGEN | ORIGINAL LIME-REQUIREMENT OF CaO PER A | LIME - REQUIREMENT AT END OF CaO PER A | DUPLICATE OF CaO PER A | AVERAGE LIME-REQUIREMENT OF CaO PER A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | *Norfolk sandy loam* | | | |
| | *gms.* | *gms.* | *gms.* | *per cent* | *per cent* | *per cent* | *gm.* | *pounds* | *pounds* | *pounds* | *pounds* |
| Check (Minerals) | 17.9 | 18.9 | 18.4 | 1.19 | 1.24 | 1.22 | 0.22 | 2,300 | 2,500 | 2,600 | 2,600 |
| 5.9 grams, 20–40 mesh | 18.9 | 22.2 | 20.6 | 1.63 | 1.75 | 1.69 | 0.35 | 2,300 | 1,600 | 1,700 | 1,700 |
| 5.9 grams, 60–80 mesh | 23.7 | 26.9 | 25.3 | 2.04 | 2.06 | 2.05 | 0.52 | 2,300 | 1,000 | 1,300 | 1,200 |
| 5.9 grams, 100–200 mesh | 23.3 | 26.0 | 24.7 | 2.04 | 2.06 | 2.05 | 0.51 | 2,300 | 1,000 | 1,000 | 1,000 |
| 5.9 grams, 200 + mesh | 26.5 | 27.3 | 26.9 | 2.10 | 2.09 | 2.10 | 0.56 | 2,300 | 700 | 1,000 | 900 |
| 3.3 grams, CaO | 29.1 | 30.0 | 29.6 | 2.00 | 2.00 | 2.00 | 0.59 | 2,300 | 900 | 1,000 | 1,000 |
| | | | | | | | | *Wooster silt loam* | | | |
| Check (Minerals) | 4.9 | 5.6 | 5.3 | 1.48 | 1.37 | 1.43 | 0.08 | 4,000 | 4,100 | 4,000 | 4,100 |
| 10.9 grams, 20–40 mesh | 7.3 | 10.4 | 8.9 | 1.86 | 1.88 | 1.87 | 0.35 | 4,000 | 2,700 | 2,700 | 2,700 |
| 10.9 grams, 60–80 mesh | 18.1 | 20.5 | 19.3 | 1.97 | 1.93 | 1.95 | 0.38 | 4,000 | 100 | 300 | 200 |
| 10.9 grams, 100–200 mesh | 21.0 | 23.4 | 22.2 | 1.86 | 1.75 | 1.81 | 0.40 | 4,000 | 200 | 200 | 200 |
| 10.9 grams, 200 + mesh | 20.9 | 21.8 | 21.4 | 1.72 | 1.81 | 1.77 | 0.38 | 4,000 | 400 | 600 | 500 |
| 6.1 grams, CaO | 20.3 | 21.9 | 21.1 | 1.64 | 1.67 | 1.66 | 0.35 | 4,000 | 200 | 200 | 200 |
| | | | | | | | | *Carrington silt loam* | | | |
| Check (Minerals) | 26.9 | 18.8 | 22.9 | 2.01 | 2.00 | 2.01 | 0.46 | 3,700 | 3,800 | 3,700 | 3,800 |
| 10.0 grams, 20–40 mesh | 28.0 | 30.2 | 29.1 | 1.89 | 1.85 | 1.87 | 0.54 | 3,700 | 2,400 | 2,800 | 2,600 |
| 10.0 grams, 60–80 mesh | 26.3 | 30.1 | 28.2 | 2.06 | 2.12 | 2.09 | 0.59 | 3,700 | 1,200 | 1,900 | 1,600 |
| 10.0 grams, 100–200 mesh | 25.6 | 32.9 | 29.3 | 2.17 | 2.14 | 2.16 | 0.64 | 3,700 | 100 | 100 | 100 |
| 10.0 grams, 200 + mesh | 31.5 | 33.6 | 32.6 | 2.17 | 2.21 | 2.19 | 0.71 | 3,700 | 100 | 100 | 100 |
| 5.6 grams, CaO | 30.7 | 39.5 | 35.1 | 2.04 | 2.03 | 2.04 | 0.72 | 3,700 | 300 | 500 | 400 |
| | | | | | | | | *Cumberland silt loam* | | | |
| Check (minerals) | 7.6 | 7.7 | 7.7 | 1.41 | 1.41 | 1.41 | 0.11 | 3,400 | 4,000 | 4,000 | 4,000 |
| 9 grams, 20–40 mesh | 10.0 | 10.6 | 10.3 | 1.48 | 1.42 | 1.45 | 0.15 | 3,400 | 2,700 | 2,700 | 2,700 |
| 9 grams, 60–80 mesh | 24.7 | 12.7 | 18.7 | 2.07 | 2.04 | 2.06 | 0.39 | 3,400 | 1,900 | 1,500 | 1,700 |
| 9 grams, 100–200 mesh | 18.8 | 19.4 | 19.1 | 1.92 | 1.93 | 1.93 | 0.37 | 3,400 | 1,100 | 1,100 | 1,100 |
| 9 grams, 200 + mesh | 19.7 | 20.7 | 20.2 | 2.00 | 2.04 | 2.02 | 0.41 | 3,400 | 900 | 700 | 800 |
| 5 grams, CaO | 13.3 | 18.6 | 16.0 | 2.05 | 2.04 | 2.05 | 0.33 | 3,400 | 1,100 | 1,600 | 1,400 |

TABLE 2

*The effect of fineness of division of pulverized limestone on crimson clover and lime-requirement*

| TREATMENT | WEIGHT OF CROP | DUPLICATE | AVERAGE | NITROGEN | NITROGEN | AVERAGE NITROGEN | TOTAL NITROGEN |
|---|---|---|---|---|---|---|---|
| *Sierra sandy loam* | | | | | | | |
| | *grams* | *grams* | *grams* | *per cent* | *per cent* | *per cent* | *gram* |
| Check (Minerals) | 32.3 | 37.0 | 34.7 | 2.06 | 2.01 | 2.04 | 0.71 |
| 2.6 grams, 20–40 mesh | 37.0 | 41.5 | 39.8 | 1.94 | 1.92 | 1.93 | 0.77 |
| 2.6 grams, 60–80 mesh | 44.6 | 45.3 | 45.0 | 1.92 | 1.90 | 1.91 | 0.86 |
| 2.6 grams, 100–200 mesh | 36.5 | 38.0 | 37.2 | 1.68 | 1.70 | 1.69 | 0.63 |
| 2.6 grams, 200 + mesh | 32.7 | 33.0 | 32.9 | 1.93 | 1.88 | 1.91 | 0.63 |
| 1.46 grams, CaO | 31.0 | 27.8 | 29.4 | 1.80 | 1.77 | 1.79 | 0.53 |
| *Portsmouth acid muck* | | | | | | | |
| Check (minerals) | 0.2 | 0.3 | 0.3 | 2.42 | 2.43 | 2.43 | 0.01 |
| 2 grams, 20–40 mesh | 18.9 | 18.5 | 18.7 | 1.66 | 1.62 | 1.64 | 0.31 |
| 2 grams, 60–80 mesh | 21.9 | 22.0 | 22.0 | 2.04 | 2.10 | 2.07 | 0.46 |
| 2 grams, 100–200 mesh | 23.7 | 23.9 | 23.8 | 2.12 | 2.14 | 2.13 | 0.51 |
| 2 grams, 200 + mesh | 22.9 | 25.1 | 24.0 | 2.24 | 2.20 | 2.22 | 0.53 |
| Commercial, 200 mesh | 23.2 | 24.2 | 23.7 | 2.10 | 2.04 | 2.07 | 0.49 |

## *Discussion*

To facilitate the discussion of the results recorded in tables 1 and 2, a summary is presented in table 3.

Table 3 shows the relative effect of fineness of division of pulverized limestone and burnt lime on crop yield on five typical soils. It will be observed that in the case of the Norfolk sandy loam an increase in fineness of division was accompanied (with one exception) by an increase in crop yield. The 200-mesh gave a yield almost one-third greater than the 20-mesh limestone. On this soil burnt lime proved superior to even the finest grade of limestone. The Wooster silt loam likewise exhibits an increase in crop yield with an increase in fineness of division of pulverized limestone up to the finest grade. It will be noted that the burnt lime, as well as the latter, falls below the yield obtained with 100-mesh limestone. This may be interpreted as indicating that the 200-mesh limestone and burnt lime were especially active in the neutralizing of the soil acidity at the outset of the experiment, but permitted a greater subsequent accumulation of acidity. The Carrington silt loam responded to an increase in fineness of division of pulverized limestone which was responsible for an increase in crop yield (with one exception). The burnt lime proved superior to all grades of limestone on this heavy soil rich in organic matter. The Cumberland silt loam otherwise shows very strikingly that an increase in fineness of division of pulverized limestone is accompanied by an increase in crop yield. In this case the 200-mesh proved to be doubly

as efficient as the 20-mesh limestone. The burnt lime was slightly inferior to the former. Thus, averaging the results for these soils in the last column of table 3, it will be seen that with an increase in fineness of division of pulverized limestone there was a proportional increase in crop yield. Furthermore the 200-mesh proved to be fully one-third again as valuable as the 20-mesh limestone. Practically all the forms of lime gave double the yield

TABLE 3

*Summary showing relative effect of fineness of division of pulverized limestone and CaO on the crop yield, total nitrogen in grams, and decrease in per cent of lime-requirement of five typical soils*

| TREATMENT | NORFOLK SANDY LOAM | WOOSTER SILT LOAM | CARRINGTON SILT LOAM | CUMBERLAND SILT LOAM | SIERRA SANDY LOAM* | AVERAGE |
|---|---|---|---|---|---|---|
| | | | *Crop yield* | | | |
| Check | 100 | 100 | 100 | 100 | 100 | 100 |
| 20–40 mesh | 112 | 357 | 127 | 134 | 115 | 183 |
| 60–80 mesh | 138 | 364 | 123 | 243 | 130 | 217 |
| 100–200 mesh | 134 | 419 | 130 | 248 | 108 | 233 |
| 200 + mesh | 146 | 404 | 142 | 262 | 95 | 239 |
| CaO | 161 | 398 | 153 | 208 | 85 | 230 |
| | | | *Total nitrogen in grams* | | | |
| Check | 100 | 100 | 100 | 100 | 100 | 100 |
| 20–40 mesh | 159 | 438 | 117 | 136 | 108 | 213 |
| 60–80 mesh | 236 | 475 | 128 | 355 | 121 | 299 |
| 100–200 mesh | 231 | 500 | 139 | 336 | 89 | 302 |
| 200 + mesh | 255 | 475 | 154 | 373 | 89 | 314 |
| CaO | 268 | 438 | 157 | 300 | 75 | 291 |
| | | | *Decrease in lime-requirement* | | | |
| Check | 100 | 100 | 100 | 100 | | 100 |
| 20–40 mesh | 135 | 134 | 132 | 133 | | 134 |
| 60–80 mesh | 154 | 195 | 158 | 158 | | 166 |
| 100–200 mesh | 162 | 195 | 197 | 173 | | 181 |
| 200 + mesh | 165 | 188 | 197 | 180 | | 183 |
| CaO | 162 | 151 | 189 | 165 | | 167 |

* Not averaged.

obtained on the unlimed soils. There is little choice between the yields obtained with burnt lime and 200-mesh limestone since each was superior in two of the four instances. Obviously, the chemical and physical nature of the soil, together with its content of organic matter, are the determining factors in the interpretation of such results.

The Sierra sandy loam which was originally alkaline presented a peculiar phenomenon, namely, the coarser limestones which act more slowly gave an

increased crop yield while the finer material and burnt lime were responsible for a decrease. The interpretation which many placed upon these facts is that the more rapidly-acting forms of lime cause certain chemical transformations to take place in the soil which prove unfavorable to crop growth. The results obtained with Portsmouth acid muck are presented in table 2. Here again it is evident that an increase in fineness of division of pulverized limestone is accompanied by an increase in crop yield. It is interesting to note that with the commercial product used ("Edison Pulverized Limestone," 80 per cent of which is guaranteed to pass a 200-mesh sieve) the crop yield was identical with that obtained with 100 to 200-mesh material, thereby fulfilling its guarantee by crop yield as well as mechanical analysis.

The importance of the nitrogen content of a leguminous crop is universally recognized alike for its feeding value and its fertilizing value in a cover crop. Summary table 3 shows the relative effect of fineness of division of pulverized limestone and burnt lime on the total nitrogen (in grams) of crimson clover. It is apparent that in practically all instances the crop yield is paralleled by the total nitrogen content; that is, with an increase in fineness of division of pulverized limestone there is an increase in the total nitrogen content of the crop. This is well illustrated in figure 1.

Undoubtedly, a highly essential consideration in the choice of the different forms of lime (aside from the paramount one of cost) is the power to neutralize soil acidity. In the present experiment, the Veitch lime-requirement of the various soils was determined at the outset, as previously recorded. After the crops had been harvested representative samples were obtained from each pot and the lime-requirement again determined. In this manner it was possible to obtain an index as to the reduction in the lime-requirements of these soils by the different forms of lime employed. While the Veitch method is open to objection for this purpose because of the fact that any residue of limestone would be soluble to the degree of its fineness and thus artificially weight the results, nevertheless, this method was adopted since it is so widely used and great accuracy is not imperative. It is, then, interesting to note that with but few exceptions an increase in fineness of division of pulverized limestone is responsible for a proportional decrease in the lime-requirements of the soils under consideration. As shown in table 3, the 200-mesh proved to be two and one-half times as effective as either the 60-mesh limestone or burnt limestone. The explanation previously suggested may be offered again in this connection, namely, the burnt lime may have been much more efficient in reducing the lime-requirement initially, but that it was used up so rapidly as to allow a subsequent development of acidity. In general the decrease in lime-requirement as a result of fineness of division is quite closely correlated with crop yield and total nitrogen content as shown in figure 1.

Thus the points which have been established in this experiment may be summarized as follows:

1. Soils representative of extensive fertile areas namely, Norfolk and Sierra sandy loams, Carrington, Wooster and Cumberland silt loams and Portsmouth acid muck, when growing crimson clover in pots, produced similar results when treated with limestone pulverized 20 to 40, 60 to 80, 100 to 200, finer than 200-mesh and burnt lime (c.p.).

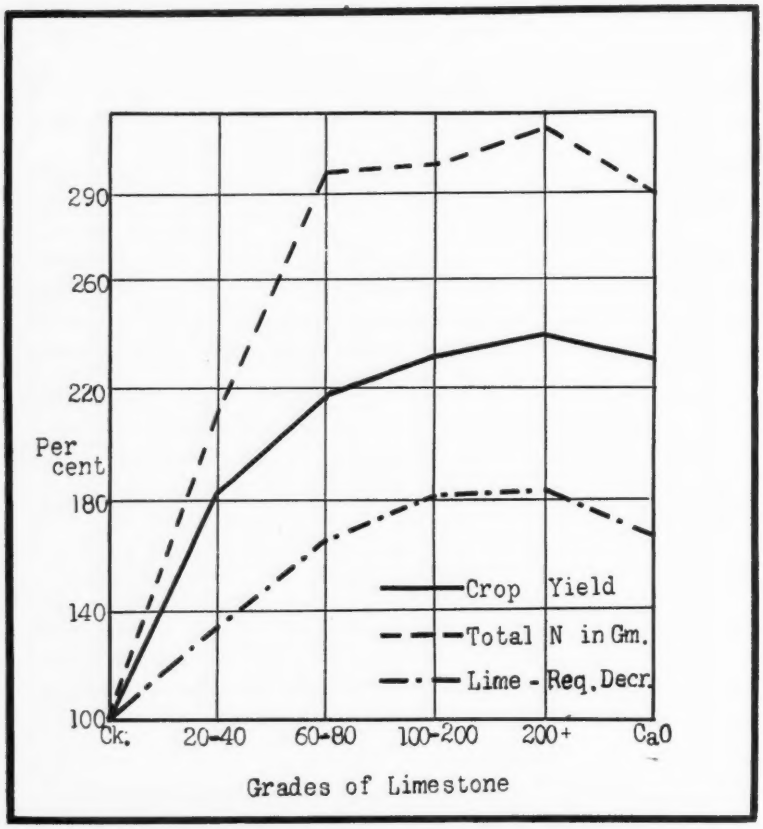



FIG. 1. DIAGRAM SHOWING THE EFFECT OF DIFFERENT DEGREES OF NITROGEN, IN GRAMS, AND THE DECREASE IN LIME-REQUIREMENT OF SEVERAL SOILS, EXPRESSED IN PER CENT

2. An increase in fineness of division of pulverized limestone was accompanied by a proportional increase in crop yield and total nitrogen content and a corresponding decrease in lime-requirement, a close correlation existing between these factors.

3. Two-hundred-mesh proved one-third again as effective in influencing crop yield as 20-mesh limestone, and more than twice as effective in reducing the lime-requirement of soils.

4. There was little choice between burnt lime and 200-mesh limestone, though in general the latter appeared preferable. Burnt lime proved superior to coarse limestone.

## THE RATE OF NEUTRALIZATION OF SOIL ACIDITY AS INFLUENCED BY DIFFERENT DEGREES OF FINENESS OF PULVERIZED LIMESTONE

In view of the enhanced effects accruing from the increased fineness of division of pulverized limestone, the practical question arose regarding the value of the different grades of pulverized limestone and burnt lime with regard to the rate of neutralization of soil acidity. Obviously, this is of great significance to the farmer who is seeking to neutralize the soil acidity so that a crop may be planted without undue delay. Since no previous work bearing directly upon this point could be found it became necessary to devise a method for measuring the rate of neutralization of acidity in soil treated with different grades of limestone and burnt lime. The Veitch method hardly lent itself to this purpose because of the fact that the water-bath treatment would result in different quantities of residual limestone going into solution, thereby invalidating any comparative results. Therefore, it was deemed advisable to measure the rate of neutralization by the carbon dioxide evolved. This was accomplished by adding a known amount of limestone to the soil (sufficient to satisfy the Veitch lime-requirement) which was calculated to its carbon dioxide content. At certain intervals the carbon dioxide remaining in the soil was determined, the loss of carbon dioxide compared with the original carbon dioxide content representing the amount of limestone which had been used up in neutralizing the soil acidity. The method employed is virtually a modification of that used by Tacke (92) and Truog (108), the apparatus being arranged as in figure 2.[2]

The procedure was as follows:

Ten grams of soil were placed in the 250-cc. Florence Flask (*A*) and 15 cc. of HCl (1: 3) run in through the separatory funnel (*B*). Enough boiling water was then added to make up a volume of about 75 cc. and the solution heated up to the boiling point in two minutes and kept there for one minute. The heat applied with a Bunsen flame was regulated in such a manner as to keep the rate at which the bubbles passed through the tower of beads (*D*) approximately constant and not very rapid. The tower (*D*) held a measured quantity of $Ba(OH)_2$ and a few drops of phenolphthalein, as did the 200-cc. Erlenmeyer flask at *E* which served as a safety flask for any carbon dioxide which might not be held at *D*. After heating for three minutes the pinchcock at *C* was closed and the flask *A* filled with boiling water from an inverted wash bottle. In order to do this properly it was usually necessary to suck on the rubber tube at *G* while cautiously opening the pinchcock at *C*. Any sudden changes in room temperature or air currents from open windows, etc., must be guarded against. When the water mounted to *F* the stop-cock at that point was closed; thus all the carbon dioxide present in the Florence flask has been displaced by boiling water. The barium hydrate solution in *D* (standardized so that 1 cc. was equivalent to 0.23 mgm.

[2] The writer is indebted to Mr. R. E. Curtis for his valuable suggestions in this connection.

of $CO_2$) was then emptied into a funnel placed in a 500-cc. Erlenmeyer flask. The beads remained on the funnel and were thoroughly washed with boiling water, as was the chamber *D* and the flask *E*, the contents of which also had been emptied. The solution of $Ba(OH)_2$ was then titrated with oxalic acid equal in strength to the $Ba(OH)_2$. Thus the amount of $Ba(OH)_2$ which was used in absorbing the carbon dioxide evolved was determined and the latter calculated accordingly. It might be mentioned that when small quantities of $Ba(OH)_2$ were placed in *D* enough $CO_2$-free water was added to bring the volume just below the connecting tube, and a small quantity of water was added also to *E*, so that the tube dipped below the liquid. The ease in manipulation and the fact that it required only ten to fifteen minutes to complete a determination were primary considerations in the adoption of this method. The duplicates checked quite closely.

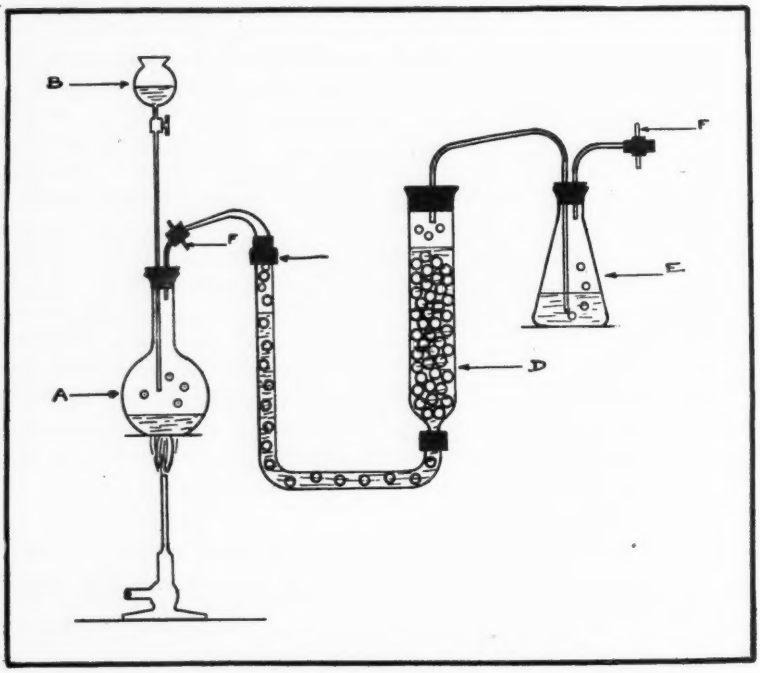



FIG. 2. DIAGRAM OF APPARATUS USED IN DETERMINING CARBON DIOXIDE IN SOIL

The following investigators have considered the solubility of calcium carbonate. Cameron and Bell (15), Holleman (43), Le Blanc and Novotny (59), Schloessing (97), Treadwell and Reuter (107), Warington (114), Whipple and Mayer (116), while Gerlach (35) has compared the solubility of limestone pulverized to different degrees of fineness. With regard to the difference in the number of particles per cubic foot of limestone when pulverized to different grades, the following data have been obtained through the courtesy of Dr. H. E. Kieffer. One cubic foot of limestone will make 64,000,000 pieces which will

pass a 12-mesh sieve, and 512,000,000,000 pieces which will pass a 200-mesh sieve; thus the finer grinding in this case means 8000 times the number of particles obtained with the coarser grinding. Or calculating it in another way, 1 ton of 12-mesh limestone when applied to the soil would give 17,337 pieces for each square foot, while 1 ton of 200-mesh limestone would give 13,869,051 pieces per square foot. The importance of this both from the point of uniform distribution and increased solubility cannot be over-estimated.

The influence of calcium carbonate on organic matter has been the subject of investigations by Frear (30), Kossowitch (55), Lemmerman (60), Peterson (89) and Wollny (121). It is evident that the organic matter of the soil would be attacked in varying degrees by different grades of pulverized limestone.

The present experiment was carried out first with Norfolk sandy loam and Penn clay loam and later with Sassafras sandy loam. Five-pound glazed earthenware pots were filled with 1500 grams of Norfolk sandy loam and 1200 grams of Penn clay loam, respectively. The lime-requirement of the former, according to the Veitch method, was 2200 pounds and the latter 1100 pounds of CaO per acre. Limestone of the different grades previously referred to and burnt lime (c.p.) were added in sufficient amount to neutralize the lime-requirements of the soils to be treated. The amount of limestone added was calculated on the basis of 90 per cent purity. Each treatment was carried out in duplicate, and untreated soils served as checks. The pots were tared, filled with the proper quantity of soil, 20 cc. of a soil infusion (known to contain the more important soil organisms in abundance) were added and enough distilled water to bring the soils up to their respective optimum moisture contents, namely, 13 per cent for the Norfolk sandy loam and 26 per cent for the Penn clay loam. The pots were supplied with glass covers to prevent evaporation and incubated in a dark room at 20°C. for 7 weeks. Moisture was added as required to the soils uniform throughout the duration of the experiment. At the end of 3 weeks, the soil in each pot was carefully mixed and a representative sample of 100 grams removed, dried and analysed for carbon dioxide content by the method previously described. The same procedure was repeated at the end of 7 weeks.

In table 4 will be found the amounts of $CO_2$ present, in the soils variously treated at the end of 3 weeks. The last column of the table was calculated in the following manner. The amount of limestone applied per 10 grams of Norfolk sandy loam was equivalent to 5.7 mgm. of $CO_2$. The check soils indicated the presence of 3.8 mgm. of $CO_2$, therefore, the total $CO_2$ content of each soil was 9.5 mgm. The $CO_2$ found to be present in 10 grams of soil in each pot after 3 weeks was then subtracted from 9.5 mgm. of $CO_2$ and the remainder recorded the mgm. of $CO_2$ used (calcium carbonate) in neutralizing soil acidity. It is evident from the results secured with both Norfolk sandy loam and Penn clay loam that an increase in fineness of division of pulverized limestone was accompanied by a proportional increase in the amount of calcium carbonate used up in neutralizing the acidity of the soil. The chief

point of interest, however, lies in the fact that the limestone finer than 60-mesh had neutralized the soil acidity at the end of 3 weeks, while this was not the case with the 20-mesh product. It appears that the finer grades of limestone, as well as the burnt lime (which had also neutralized the soil acidity in this period of time) had begun to attack the organic matter of the soil.

The results obtained at the end of 7 weeks as shown in table 5 indicated that the coarse limestone had very nearly succeeded neutralizing the soil acidity in the Norfolk sandy loam but was not quite so effective in the Penn clay loam. The other grades of limestone exhibited practically no differences other than those already noted. Thus from the foregoing data it appears that the finer grades of limestone neutralize the soil acidity in 3

TABLE 4

*The effect of fineness of division of pulverized limestone on the neutralization of acidity as measured by $CO_2$ present after three weeks' incubation*

| TREATMENT | $CO_2$ | $CO_2$ | AVERAGE $CO_2$ | INCREASE OVER CHECK $CO_2$ USED |
|---|---|---|---|---|
| *Norfolk sandy loam* | | | | |
| | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| Check | 3.7 | 3.9 | 3.8 | |
| 20– 40 mesh | 6.3 | 7.5 | 6.9 | 2.6 |
| 60– 80 mesh | 3.1 | 3.2 | 3.2 | 6.3 |
| 100–200 mesh | 3.0 | 2.3 | 2.7 | 6.8 |
| 200 + mesh | 1.6 | 1.8 | 1.7 | 7.8 |
| CaO | 1.4 | 1.6 | 1.5 | 8.0 |
| *Penn clay loam* | | | | |
| Check | 4.6 | 4.7 | 4.7 | |
| 20– 40 mesh | 6.9 | 7.3 | 7.1 | 1.4 |
| 60– 80 mesh | 5.6 | 5.5 | 5.6 | 2.9 |
| 100–200 mesh | 4.1 | 4.0 | 4.1 | 4.4 |
| 200 + mesh | 3.2 | 3.9 | 3.6 | 4.9 |
| CaO | 2.8 | 2.4 | 2.6 | 5.9 |

weeks even in soils of such widely divergent types as those employed in the present instance. In this respect they proved to be as efficient as burnt lime. The coarser limestone, on the other hand, required more than double this period of time to neutralize the soil acidity. Wilkins (120), in unpublished data, has shown that in a given period of time an increase in fineness of division of pulverized limestone is responsible for an increased production of $CO_2$ and nitrates.

In order to obtain further corroboration of the points brought out in the preceding experiment, the latter was repeated under similar conditions with another soil, namely, Sassafras sandy loam having a lime-requirement of 3300 pounds of CaO per acre. An additional feature was the use of two and one-

half times the amount of 20-mesh limestone as required in order to determine whether larger amounts of coarser limestone would be as effective as smaller amounts of finer material in a short period of time.[3] This seemed advisable in view of the fact that the pot experiments previously referred to indicated that the 200-mesh limestone was most effective in neutralizing acidity and further because the same proportion was maintained with regard to the rate of time required for neutralization.

TABLE 5

*The effect of fineness of division of pulverized limestone on the neutralization of acidity as measured by $CO_2$ present after seven weeks' incubation*

| TREATMENT | $CO_2$ | $CO_2$ | AVERAGE $CO_2$ | INCREASE OVER CHECK $CO_2$ USED |
|---|---|---|---|---|
| *Norfolk sandy loam* | | | | |
| | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| Check | 2.4 | 2.7 | 2.6 | |
| 20– 40 mesh | 3.5 | 4.4 | 4.0 | 4.3 |
| 60– 80 mesh | 3.0 | 2.6 | 2.8 | 5.5 |
| 100–200 mesh | 1.7 | 1.8 | 1.8 | 6.5 |
| 200 + mesh | 1.8 | 2.1 | 2.0 | 6.3 |
| CaO | 1.6 | 1.6 | 1.6 | 6.7 |
| *Penn clay loam* | | | | |
| Check | 3.8 | 3.7 | 3.8 | |
| 20– 40 mesh | 6.0 | 5.8 | 5.9 | 1.7 |
| 60– 80 mesh | 4.2 | 4.7 | 4.5 | 3.1 |
| 100–200 mesh | 2.6 | 2.9 | 2.8 | 4.8 |
| 200 + mesh | 2.6 | 2.9 | 2.8 | 4.8 |
| CaO | 1.7 | 1.7 | 1.7 | 5.9 |

The results as recorded in table 6 are substantially the same as those obtained with the other soil types, namely, with an increase in fineness of pulverized limestone there is an increased amount of calcium carbonate used in neutralizing soil acidity. Concerning the salient point of this investigation it is apparent that the 200-mesh has required but two weeks to effect neutralization, the 60-mesh requires three to four weeks, while the 20-mesh limestone requires a still longer period of time. The burnt lime, as might be anticipated, neutralized the soil acidity in the first week and must be considered as more rapid in its activity than even the finest grade of limestone used. Glancing at the results obtained with the increased quantity of coarse limestone, it will be noted that it proved fully as effective as the finest limestone.

Thus the preference for finer over coarser limestone would resolve itself chiefly to a question of economy in purchase, hauling, distribution, etc. The

[3] The writer is indebted to Mr. C. M. Haensler for suggesting this point.

TABLE 6

*The rate of neutralization of acidity by different degrees of fineness of pulverized limestone in Sassafras sandy loam*

| TREATMENT | $CO_2$ | $CO_2$ | AVERAGE $CO_2$ | $CO_2$ USED |
|---|---|---|---|---|
| | *After one week* | | | |
| | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| Check | 1.6 | 2.0 | 1.8 | |
| 20–40 mesh | 5.9 | 6.4 | 6.2 | 5.1 |
| 2.5 × 20–40 mesh | 16.2 | 16.7 | 16.5 | 7.2 |
| 60– 80 mesh | 5.9 | 6.4 | 6.2 | 5.1 |
| 100–200 mesh | 5.7 | 5.9 | 5.8 | 5.5 |
| 200 + mesh | 5.0 | 4.8 | 4.9 | 6.4 |
| CaO | 4.3 | 4.1 | 4.2 | 7.1 |
| | *Second week* | | | |
| Check | 1.6 | 2.1 | 1.9 | |
| 20–40 mesh | 6.8 | 7.0 | 6.9 | 4.5 |
| 2.5 × 20–40 mesh | 15.2 | 15.3 | 15.3 | 8.4 |
| 60– 80 mesh | 5.5 | 5.9 | 5.7 | 5.7 |
| 100–200 mesh | 4.6 | 4.5 | 4.6 | 6.8 |
| 200 + mesh | 2.6 | 2.9 | 2.8 | 8.6 |
| CaO | 2.6 | 2.8 | 2.7 | 8.7 |
| | *Third week* | | | |
| Check | 1.9 | 1.9 | 1.9 | |
| 20– 40 mesh | 7.0 | 7.4 | 7.2 | 4.2 |
| 2.5 × 20–40 mesh | 15.7 | 15.0 | 15.4 | 8.5 |
| 60– 80 mesh | 5.5 | 5.2 | 5.4 | 6.0 |
| 100–200 mesh | 4.6 | 5.2 | 4.9 | 6.5 |
| 200 + mesh | 4.3 | 3.8 | 4.1 | 7.3 |
| CaO | 2.9 | 3.1 | 3.0 | 8.4 |
| | *Fourth week* | | | |
| Check | 1.0 | 1.0 | 1.1 | |
| 20– 40 mesh | 6.5 | 6.4 | 6.5 | 4.1 |
| 2.5 × 20–40 mesh | 15.0 | 14.7 | 14.9 | 8.8 |
| 60– 80 mesh | 5.2 | 5.1 | 5.2 | 5.4 |
| 100–200 mesh | 3.4 | 3.5 | 3.5 | 7.1 |
| 200 + mesh | 2.5 | 2.7 | 2.6 | 8.0 |
| CaO | 2.6 | 2.6 | 2.6 | 8.0 |

points established in the preceding experiments may be summarized as follows:

1. An apparatus is described for measuring the rate of neutralization of acidity by different degrees of fineness of pulverized limestone.

2. It was found that with Norfolk sandy loam as well as with Penn clay loam an increase in fineness of division of pulverized limestone was responsible for an increase in amount of limestone used up in the neutralization of soil acidity.

3. At the end of three weeks the limestone finer than 60 to 80-mesh had neutralized the acidity in both soils, while 20 to 40-mesh limestone required something more than seven weeks to accomplish the same result. The finer mesh limestone was as effective in three weeks as burnt lime.

4. With Sassafras sandy loam, the above results were corroborated by weekly determinations. In this soil the burnt lime neutralized the acidity in one week, the 200-mesh limestone required two weeks, the 60-mesh three to four weeks and the 20-mesh even a longer period of time.

5. Twenty-mesh limestone used in quantities two and one-half times as great as fine limestone proved fully as effective as the latter in three weeks

## THE FINENESS OF DIVISION OF PULVERIZED LIMESTONE AS INFLUENCING THE BACTERIOLOGICAL ACTIVITIES IN THE SOIL

No study of a soil amendment could be considered adequate without an investigation concerning its effect upon the activities of the soil microorganisms. This is especially true of lime since it determines to such a marked degree the reaction of the soil. So far as the writer has been able to ascertain only one experiment has been reported which deals with the effect of fineness of division of pulverized limestone upon soil biological activities. This work has been done at the Pennsylvania Agricultural Experiment Station (117), where a study was being made of the effects of pure and magnesian limestone upon the formation of nitrate as compared with equivalent amounts of burned lime.[4] It was found that an increase in the fineness of division of pulverized limestone caused an increase in nitrification. Burnt lime proved superior to any grade of limestone used.

The fact that in general lime increases the number of soil organisms has been observed by Brown (11), Engberding (20), Hutchinson (46), and Miller (74). Again the evidence of Brown (11), Hutchinson (41), MacLennan (47, 48), Kelly (52), Koch (54), Krueger (57), Lipman (62, 63), Lipman, Brown and Owen (66, 67), Paterson and Scott (83), and Peck (87), points to the fact that lime increases ammonification. The stimulating effect of lime upon nitrification has been investigated by Brown (11), Densch (17), Fischer (24), Fittbogen (26), Fraps (28), Fred (52), Kelley (51, 52), Lyon and Bizzell (67), Lipman (62), McBeth and Wright (71), Miller (73), Müller and Weiss (79), Owen (82), Peck (86), Scales (96), Wilkins (120), and Wright (123). Denitrification studies have been performed by Lemmerman and Fischer (60), Lipman (62),

[4] Burgess has found that nitrification is increased by fine material compared with coarse. Burgess, P. S., Nitrification as a measure of the availability of different forms of calcium carbonate when employed as correctors of soil acidity. *In* Soil Sci. (soon to appear).

and Vogel (112). Finally, the influence of lime on nitrogen-fixation has been investigated by Brown (11), Engberding (20), Hoffman and Hammer (42), Koch (55), Krainskii (56), Krueger (57), Lipman (62), and Vogel (112).

In the light of the interesting differences obtained in crop yield and neutralization of soil acidity it seemed advisable to carry out some experiments to determine the effect of different degrees of fineness of pulverized limestone on ammonification, nitrification and nitrogen-fixation. An attempt was made to correlate these biological activities with the rate of neutralization of soil acidity in the following manner. Norfolk sandy loam and Penn clay loam were incubated for seven weeks in 5-pound glazed earthenware pots with different grades of pulverized limestone and burnt lime, under the same conditions as described in the previous experiments. Ten cubic centimeters of an infusion made from soil known to contain active ammonifying and nitrifying bacteria; as well as 10 cc. of an infusion made from "Alphano humus," known to contain active nitrogen-fixing organisms, were added to each pot of soil. At the end of three weeks, when the soils were first sampled to determine the rate of neutralization of acidity, 5-grams portions of moist soil were immediately introduced into sterile flasks of media for the measurement of biological activities. These were prepared as follows, accordingtothe methods described by Löhnis and Green (66).

A soil extract was prepared from each soil by adding 2 volumes of distilled water to 1 volume of soil and autoclaving in cotton-plugged Erlenmeyer flasks at 15 pounds pressure for fifteen minutes. After cooling and settling the supernatant liquid was filtered through several thicknesses of filter paper until a clear extract was obtained. To this was added 0.05 gram of $K_2HPO_4$ per litre. For ammonification, 50 cc. of this solution to which had been added 0.5 gram of peptone per flask were inoculated (in a 750-cc. Florence flask) with a 5-gram sample of soil and incubated at 23°C. for three days. At the end of this time the contents of the flasks were distilled with magnesium oxide and titrated in the usual manner. For nitrification, to 50 cc. of the extract of the soil type used as inoculum were added 50 mgm. of ammonium sulfate and 1 gram of calcium carbonate (c.p.). These flasks were incubated after inoculation with 5 grams of soil for four weeks. The nitrates were determined colorimetrically by the phenoldisulphonic acid method. For nitrogen-fixation 2 grams of dextrose and 2 grams of calcium carbonate were added to 50 cc. of soil extract and incubated for three weeks, at the end of which time they were Kjeldahled. Suitable checks were carried in duplicate in all instances and all the determinations on each soil were made in duplicate. Since the soils received duplicate treatments of lime the possibilities for error were reduced to a minimum.

The results of the ammonification of peptone after three weeks' incubation of soil with lime are recorded in table 7.

It will be seen that in both the Norfolk sandy loam and the Penn clay loam (with one exception) there is an increase in ammonification with an increase in fineness of division of pulverized limestone. It is of interest to note that while the differences in ammonification resulting from the finer grades of limestone are comparatively slight, there is a striking difference between the coarse and the finer limestone in both soils. In the Norfolk sandy loam,

for example, there was an increase of only 0.1 mgm. of nitrogen over the check for the 20-mesh as compared with 7.8 mgm. of nitrogen for the 200-mesh limestone. While in the Penn clay loam it was 0.3 mgm. of nitrogen for the 20-mesh as against 3.3 mgm. of nitrogen for the 200-mesh limestone. The burnt lime was more effective than the finest limestone in its influence on this biological activity. As might be expected from the more advantageous environmental conditions, the Norfolk sandy loam was responsible for greater differences between treatments than was the Penn clay loam.

Regarding these results in their entirety, it will be remembered that they are in accord with those obtained in determining the rate of neutralization of the different grades of pulverized limestone.

TABLE 7

*The effect of fineness of division of pulverized limestone on the ammonification of peptone in solution (soil incubated with lime three weeks)*

| TREATMENT | NITROGEN | NITROGEN | AVERAGE NITROGEN | INCREASE IN NITROGEN |
|---|---|---|---|---|
| *Norfolk sandy soil* | | | | |
| | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| Check | 26.0 | 26.1 | 26.1 | |
| 20– 40 mesh | 26.2 | Lost | 26.2 | 0.1 |
| 60– 80 mesh | 31.8 | 31.7 | 31.8 | 5.7 |
| 100–200 mesh | 32.9 | 32.6 | 32.8 | 6.7 |
| 200 + mesh | 32.0 | 35.7 | 33.9 | 7.8 |
| CaO | 35.6 | 37.3 | 36.5 | 10.4 |
| *Penn clay loam* | | | | |
| Check | 23.0 | 26.0 | 24.6 | |
| 20– 40 mesh | 23.6 | 26.1 | 24.9 | 0.3 |
| 60– 80 mesh | 27.4 | 27.4 | 27.4 | 2.8 |
| 100–200 mesh | 25.0 | 28.6 | 26.9 | 2.3 |
| 200 + mesh | 27.1 | 28.4 | 27.9 | 3.3 |
| CaO | 29.7 | 29.0 | 29.4 | 4.8 |

The results at the end of seven weeks of incubation of soil with limestone are presented in table 8. It is evident that the differences between treatments in both soils are hardly large enough to bear mention, except in so far as the 20-mesh limestone gave the highest ammonification. This again may be correlated very closely with the results obtained concerning the rate of neutralization of acidity. In other words, the finer the limestone the more rapidly it neutralizes the acidity of the soil and the more effectively it increases the process of ammonification. It will be remembered that the 20-mesh material was the only one to be found present to any extent at the end of the seven-week period; therefore, it might well be expected that the present maximum of ammonia should occur with that grade of limestone.

Because of the importance of soil reaction in influencing nitrification, one would be led to anticipate marked differences in the accumulation of nitrates as a result of treating the soil with different grades of pulverized limestone. That this is virtually the case is shown by table 9, where it will be seen that with such widely divergent soil types, the differences in treatment at the end of three weeks yield results which are in identically the same direction, namely, an increase in fineness of division of pulverized limestone is responsible for an increased accumulation of nitrates. Again the burnt lime proved superior and the 200-mesh is strikingly better than the 20-mesh limestone in influencing this important biological activity in the soil. Thus a correlation exists between the rate of neutralization and nitrification as well as ammonifica-

TABLE 8

*The effect of fineness of division of pulverized limestone on the ammonification of peptone in solution (soil incubated with lime seven weeks)*

| TREATMENT | NITROGEN | NITROGEN | AVERAGE NITROGEN |
|---|---|---|---|
| *Norfolk sandy loam* | | | |
| | *mgm.* | *mgm.* | *mgm.* |
| Check | 42.8 | 43.3 | 43.1 |
| 20– 40 mesh | 43.6 | 46.2 | 44.9 |
| 60– 80 mesh | 41.0 | 40.8 | 40.9 |
| 100–200 mesh | 41.1 | Lost | 41.1 |
| 200 + mesh | 42.8 | 41.7 | 42.3 |
| CaO | 41.8 | 43.5 | 42.2 |
| *Penn clay loam* | | | |
| Check | 32.0 | 33.2 | 32.6 |
| 20– 40 mesh | 33.5 | 32.6 | 33.1 |
| 60– 80 mesh | 32.8 | 33.3 | 33.1 |
| 100–200 mesh | 33.0 | 31.0 | 32.0 |
| 200 + mesh | 32.4 | 33.1 | 32.8 |
| CaO | 31.6 | 32.9 | 32.3 |

tion. Coleman[5] has a comprehensive discussion and bibliography dealing with nitrogen transformations in the soil. Since the accompanying data pointed indubitably to the fact that one could expect little, if any, differences in nitrification after seven weeks' incubation of soil with limestone, these determinations were discontinued.

The results obtained in the nitrogen-fixation experiments at the end of three weeks have not been tabulated for the reason that no differences worthy of note were established. For example, in the Norfolk sandy loam at the end of three weeks, the average number of milligrams of nitrogen present in the check soils was 3.37, while the amount in the variously treated soils ranged from the

[5] Doctor's Thesis, Rutgers College, New Brunswick, N. J. (1917).

above-mentioned quantity up to 3.71 mgm. of nitrogen. In the Penn clay loam the check had 4.63 mgm. of nitrogen, and the range in variation due to treatment approached 5.04 mgm. of nitrogen as a maximum. At the end of seven weeks there was a distinct increase over the check in the soils treated with limestone, as shown in table 10, but the differences between treatments were not within experimental error in the case of Norfolk sandy loam. Thus 3.8 mgm. of nitrogen were present in the check soils and 12.3 to 12.9 mgm. of nitrogen in the treated soils. In Penn clay loam, however, there is an apparent tendency for the amount of nitrogen fixed to increase as the fineness of division of pulverized limestone increased.

TABLE 9

*The effect of fineness of division of pulverized limestone on nitrification in solution (soil incubated with limestone three weeks)*

| TREATMENT | $NO_3$ | $NO_2$ | AVERAGE | INCREASE OVER CHECK |
|---|---|---|---|---|
| *Norfolk sandy loam* | | | | |
| | *p.p. m.* | *p.p. m.* | *p.p. m.* | *p.p. m.* |
| Check | 7.4 | 6.8 | 7.1 | |
| 20– 40 mesh | 7.9 | 8.2 | 7.8 | 0.7 |
| 60– 80 mesh | 8.2 | 8.2 | 8.2 | 1.1 |
| 100–200 mesh | 9.2 | 9.8 | 9.5 | 2.4 |
| 200 + mesh | 10.2 | 13.0 | 11.6 | 4.5 |
| CaO | 9.4 | 20.0 | 14.7 | 7.6 |
| *Penn clay loam* | | | | |
| Check | 7.6 | 8.4 | 8.0 | |
| 20– 40 mesh | 11.4 | 18.4 | 14.9 | 6.9 |
| 60– 80 mesh | 15.4 | 15.2 | 15.3 | 7.3 |
| 100–200 mesh | 12.6 | 22.8 | 17.7 | 9.7 |
| 200 + mesh | 20.6 | 22.0 | 21.3 | 13.3 |
| CaO | 24.0 | 28.0 | 26.0 | 18.0 |

Considering this experiment in its entirety, it has been established that both in Norfolk sandy loam and in Penn clay loam an increase in fineness of division of pulverized limestone was responsible for a corresponding increase in the rate of neutralization of acidity as well as enhanced biological activities in the soil as measured by ammonification, nitrification and nitrogen-fixation (with limitations) in solution.

In order to substantiate these points a similar experiment was performed in which Sassafras sandy loam was used in the same manner as previously described in the experiment dealing with the rate of neutralization of acidity, that is, burnt lime and the following grades of limestone were used: 20 to 40-mesh, 60 to 80-mesh, 100 to 200-mesh and finer than 200-mesh. In the present case, however, the fresh soil methods recommended by Brown (12, 13) were

employed. For ammonification, 100 grams of moist soil were thoroughly mixed in a shaker (61) with cottonseed meal or dried blood containing 155 mgm. of nitrogen, respectively, and incubated at optimum moisture content for six days at 20°C., the ammonia being determined by the magnesium oxide method at the end of this time. For nitrification 100 mgm. of ammonium sulfate were added per tumbler and incubated at optimum moisture content for thirty days at 18°C. At the end of this time the nitrates were leached out by adding 400 cc. of distilled water, some ground burnt lime was added and the whole shaken thoroughly for five minutes, filtered, and a 25-cc. aliquot taken for determination according to the colorimetric phenoldisulphonic acid method. For nitrogen-fixation 2 grams of mannite were added to 50

TABLE 10

*The effect of fineness of division of pulverized limestone on the rate of neutralization of acidity as affecting nitrogen fixation in solution after seven weeks incubation of soil with limestone*

| TREATMENT | NITROGEN | NITROGEN | AVERAGE NITROGEN |
|---|---|---|---|
| *Norfolk sandy loam* | | | |
| | *mgm.* | *mgm.* | *mgm.* |
| Check | 3.8 | 3.8 | 3.8 |
| 20– 40 mesh | 11.4 | 13.2 | 12.3 |
| 60– 80 mesh | 12.1 | 12.4 | 12.3 |
| 100–200 mesh | 12.5 | Lost | 12.5 |
| 200 + mesh | 12.6 | 13.2 | 12.9 |
| CaO | 11.5 | 13.3 | 12.4 |
| *Penn clay loam* | | | |
| Check | 4.1 | 5.4 | 4.8 |
| 20– 40 mesh | 5.2 | 6.4 | 5.8 |
| 60– 80 mesh | 7.0 | 8.5 | 7.8 |
| 100–200 mesh | 7.5 | 8.0 | 7.8 |
| 200 + mesh | 7.6 | 8.7 | 8.2 |
| CaO | 8.0 | 8.4 | 8.2 |

grams of soil and incubated at optimum moisture content for twenty-one days at 18°C. At the end of this period the soil was air-dried, pulverized and nitrogen determined by the Kjeldahl method.

The results of ammonification after one week's incubation of soil with limestone are recorded in table 11.

It will be seen that with cottonseed meal an increase in fineness of division of pulverized limestone was responsible for a corresponding increase in ammonia found. The 200-mesh is one-third more effective than the 20-mesh limestone, but burnt lime is somewhat superior to the former. The results obtained with dried blood are peculiar in that the 20- and the 60-mesh limestone yielded smaller quantities than were found in the checks, while the

finer grades of limestone and burnt lime exhibited an increase. This point will bear more complete discussion in another connection.

With cottonseed meal the results in table 11 obtained after two weeks' incubation (with one exception) show that in increase in fineness of division of pulverized limestone is accompanied by an increase in ammonification. However, the differences between treatments are not quite as pronounced as after the first week, undoubtedly because of the fact that the different grades of limestone tend to equalize, as is shown by the work on the rate of neutralization of acidity. Again the 200-mesh is markedly superior to the 20-mesh

TABLE 11

*The effect of fineness of division of pulverized limestone on the ammonification of cottonseed meal and dried blood (Sassafras sandy loam incubated with lime for one and two weeks)*

| TREATMENT | COTTONSEED MEAL | | | DRIED BLOOD | | |
|---|---|---|---|---|---|---|
| | Nitrogen | Nitrogen | Average nitrogen | Nitrogen | Nitrogen | Average nitrogen |
| *After one week's incubation* | | | | | | |
| | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| Check | 47.1 | 49.3 | 48.2 | 42.5 | 43.5 | 43.0 |
| 20– 40 mesh | 50.0 | 50.5 | 50.3 | 37.6 | 38.3 | 38.0 |
| 2.5 × 20–40 mesh | 47.8 | 51.5 | 49.7 | 38.3 | 38.9 | 38.6 |
| 60– 80 mesh | 60.4 | 56.4 | 58.4 | 32.8 | 35.2 | 34.0 |
| 100–200 mesh | 53.7 | 66.5 | 60.1 | 41.3 | 45.7 | 43.5 |
| 200 + mesh | 65.8 | 68.0 | 66.9 | 48.5 | 51.7 | 50.1 |
| CaO | 68.3 | 68.9 | 68.6 | 46.9 | 53.6 | 50.2 |
| *After two weeks' incubation* | | | | | | |
| Check | 53.1 | 54.7 | 53.9 | 56.6 | 53.3 | 55.0 |
| 20– 40 mesh | 55.7 | 54.7 | 55.2 | 47.2 | 46.7 | 47.0 |
| 2.5 × 20–40 mesh | 57.6 | 57.0 | 57.3 | 43.5 | 41.8 | 42.7 |
| 60– 80 mesh | 64.0 | 64.6 | 64.3 | 33.0 | 37.9 | 35.5 |
| 100–200 mesh | 63.7 | 61.0 | 62.4 | 36.0 | 26.6 | 31.3 |
| 200 + mesh | 65.0 | 64.6 | 64.8 | 27.4 | 27.5 | 27.5 |
| CaO | 65.6 | 66.6 | 66.1 | 30.4 | 28.6 | 29.5 |

limestone but inferior to the burnt lime. A point of interest is to be remarked in the fact that two and one-half times the usual quantity of 20-mesh limestone does not increase ammonification to any marked degree over that obtained with the normal amount, and is decidedly inferior to the 200-mesh limestone. The results with dried blood show a progressive decrease in ammonia with an increase in fineness of division of pulverized limestone. The explanation which suggests itself is that the ammonification of dried blood is actually more rapid with an increase in fineness of division of pulverized limestone but that the bacteria multiply so rapidly as to assimilate it again, so that it does not appear as ammonia when distilled with MgO, at the end of the six-day

incubation period, but is transformed into other nitrogenous compounds. This theory is substantiated by further experiments where the writer has found an increase in ammonification of dried blood with an increase in fineness of division of pulverized limestone. Furthermore, preliminary experiments were performed in which the ammonification of dried blood was accompanied by a daily measurement of carbon dixoide evolved. This was accomplished by aspirating air continuously through a stoppered bottle containing soil, which was connected with an Erlenmeyer flask containing a known quantity of standard barium hydrate. It was found that with an increase in fineness of division of pulverized limestone there was an increased production of carbon dioxide which may well be regarded as an index of decomposition effected by microbial activities. Thus it may be assumed that an increase in fineness of division of pulverized limestone actually caused an increase in the ammonification of dried blood, but that because of a subsequent assimilation of ammonia the amounts of ammonia found by distilling with magnesium oxide were comparatively small.

TABLE 12

*The effect of fineness of division of pulverized limestone on ammonification (Sassafras sandy loam incubated with lime for three weeks)*

| TREATMENT | NITROGEN | NITROGEN | AVERAGE NITROGEN |
|---|---|---|---|
| *Cottonseed meal* | | | |
| | *mgm.* | *mgm.* | *mgm.* |
| Check | 50.3 | 47.5 | 48.9 |
| 20– 40 mesh | 49.4 | 57.1 | 53.3 |
| 2.5 × 20–40 mesh | 52.6 | 54.1 | 53.4 |
| 60– 80 mesh | 62.6 | 63.2 | 62.9 |
| 100–200 mesh | 63.0 | 50.1 | 56.6 |
| 200 + mesh | 59.0 | 56.0 | 57.5 |
| CaO | 48.2 | 56.2 | 52.2 |
| *Dried blood* | | | |
| Check | 52.8 | 48.5 | 50.7 |
| 20– 40 mesh | 41.6 | 44.5 | 43.1 |
| 2.5 × 20–40 mesh | 41.9 | 40.0 | 41.0 |
| 60– 80 mesh | 28.6 | 35.0 | 31.8 |
| 100–200 mesh | 31.0 | 20.0 | 25.5 |
| 200 + mesh | 22.2 | 22.4 | 22.3 |
| CaO | 20.6 | 21.6 | 21.1 |

In tables 12 and 13 are recorded the results obtained after incubating soil with limestone for three and four weeks, respectively. With cottonseed meal as well as dried blood the general tendencies heretofore noted are again to be observed.

Considering the results obtained in the nitrification of ammonium sulfate after one week's incubation of soil with lime, it will be seen from table 14,

TABLE 13

*The effect of fineness of division of pulverized limestone on ammonification (Sassafras sandy loam incubated with limestone four weeks)*

| TREATMENT | NITROGEN | NITROGEN | AVERAGE NITROGEN |
|---|---|---|---|
| *Cottonseed meal* | | | |
| | *mgm.* | *mgm.* | *mgm.* |
| Check | 35.4 | 35.2 | 35.3 |
| 20– 60 mesh | 32.5 | 37.0 | 34.8 |
| 2.5 × 20–60 mesh | 37.4 | 39.0 | 38.2 |
| 60– 80 mesh | 41.0 | 38.2 | 39.6 |
| 100–200 mesh | 40.3 | 37.3 | 38.8 |
| 200 + mesh | 42.1 | 38.9 | 40.5 |
| CaO | 33.1 | 36.6 | 33.9 |
| *Dried blood* | | | |
| Check | 32.3 | 32.5 | 32.4 |
| 20– 60 mesh | 28.2 | 29.3 | 28.8 |
| 2.5 × 20–60 mesh | 26.7 | 28.2 | 27.5 |
| 60– 80 mesh | 24.0 | 24.6 | 24.3 |
| 100–200 mesh | 21.8 | 14.9 | 18.4 |
| 200 + mesh | 17.9 | 17.0 | 17.5 |
| CaO | 13.9 | 15.2 | 14.6 |

TABLE 14

*The effect of fineness of division of pulverized limestone on nitrification (Sassafras sandy loam incubated with limestone one and two weeks)*

| TREATMENT | NITROGEN | NITROGEN | AVERAGE NITROGEN | INCREASE OVER CHECK |
|---|---|---|---|---|
| *After one week's incubation* | | | | |
| | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| Check on soil | 0.06 | 0.06 | 0.06 | |
| Check | 0.13 | 0.12 | 0.13 | 0.07 |
| 20– 40 mesh | 0.12 | 0.17 | 0.15 | 0.09 |
| 2.5 × 20–40 mesh | 0.43 | 0.46 | 0.45 | 0.39 |
| 60– 80 mesh | 0.51 | 0.96 | 0.74 | 0.68 |
| 100–200 mesh | 1.28 | 0.70 | 0.99 | 0.93 |
| 200 + mesh | Lost | Lost | Lost | Lost |
| CaO | 0.55 | 0.23 | 0.39 | 0.33 |
| *After two weeks' incubation* | | | | |
| Check | 0.10 | 0.09 | 0.10 | 0.04 |
| 20– 40 mesh | 0.32 | 0.17 | 0.25 | 0.19 |
| 2.5 × 20–40 mesh | 2.46 | 1.48 | 1.97 | 1.37 |
| 60– 80 mesh | 4.00 | 4.90 | 4.45 | 4.39 |
| 100–200 mesh | 1.48 | 0.31 | 0.90 | 0.84 |
| 200 + mesh | 0.31 | 0.65 | 0.48 | 0.42 |
| CaO | 0.15 | 0.11 | 0.13 | 0.07 |

that an increase in fineness of division of pulverized limestone is responsible for a corresponding accumulation of nitrates. The results obtained in the subsequent weeks of incubation, as shown in tables 14 and 15—are more variable, because the moisture conditions were not strictly maintained. However, the tendency follows the general rule previously observed, namely, an increase in fineness of division of pulverized limestone is responsible for an increase in biological activities, which may be correlated with the rate of neutralization of acidity. In the present experiment the differences in nitrogen-fixation, as a result of variation in treatment, were not of sufficient magnitude to permit of any definite conclusions. The average number of milligrams of nitrogen found varied from 0.98 to 1.21. It is possible that the amount of carbohydrate used in the present instance was so excessive as to inhibit nitrogen-fixation.

In order to emphasize further the importance of fineness of division of pulverized limestone in influencing ammonification, several soils (the same

TABLE 15

*The effect of fineness of division of pulverized limestone on nitrification (Sassafras sandy loam incubated with lime for three weeks)*

| TREATMENT | NITROGEN | NITROGEN | AVERAGE NITROGEN | INCREASE OVER CHECK |
|---|---|---|---|---|
| | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| Check | 0.46 | 0.43 | 0.30 | 0.24 |
| 20– 40 mesh | 0.37 | 0.17 | 0.27 | 0.21 |
| 2.5 × 20–40 mesh | 1.96 | 1.95 | 1.95 | 1.90 |
| 60– 80 mesh | 3.01 | 2.71 | 2.86 | 2.80 |
| 100–200 mesh | Lost | 0.94 | 0.94 | 0.88 |
| 200 + mesh | 1.20 | 1.37 | 1.29 | 1.23 |
| CaO | 0.75 | 0.72 | 0.74 | 0.68 |

as previously used in growing clover in pots) were incubated with the different grades of limestone and burnt lime for one week. At the end of this time 100-gram portions from each pot received nitrogenous organic material in the form of dried blood or cottonseed meal (155 mgm. N), and were incubated at the optimum moisture content for seven days at 20°C. The results obtained with Cumberland silt loam are recorded in table 16.

Although the differences between treatments are not great, nevertheless it will be seen that in general, with both cottonseed meal and dried blood, an increase in fineness of division of pulverized limestone is accompanied by an increase in ammonia found. The burnt lime and 200-mesh limestone were equally efficient. Two and one-half times the usual quantity of 20-mesh limestone gives better results, not markedly superior to the normal application.

The results obtained with Wooster and Carrington silt loams are presented in table 16. The differences between treatments are not very marked but the finer material appears to better advantage than the coarser. Burnt lime

proved more efficient than 200-mesh limestone. The results obtained with increased quantities of 20-mesh limestone are similar to those noted where Cumberland silt loam was employed.

A general consideration of these soils of fine texture and high organic content reveals the fact that in the ammonification of nitrogenous organic ma-

TABLE 16

*The effect of fineness of division of pulverized limestone on ammonification in Cumberland, Carrington and Wooster silt loams*

| TREATMENT | COTTONSEED MEAL | | | DRIED BLOOD | | |
|---|---|---|---|---|---|---|
| | Nitrogen | Nitrogen | Average nitrogen | Nitrogen | Nitrogen | Average nitrogen |
| | *Cumberland silt loam* | | | | | |
| | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| Check | 70.0 | 70.7 | 70.4 | 78.3 | 77.4 | 77.9 |
| 20– 40 mesh | 74.9 | 69.3 | 72.1 | 64.8 | 66.6 | 65.7 |
| 2.5 × 20–40 mesh | Lost | 73.8 | 73.8 | 67.8 | 64.8 | 66.3 |
| 60– 80 mesh | 73.4 | 72.0 | 72.7 | 61.4 | 71.8 | 66.6 |
| 100–200 mesh | 73.8 | 74.3 | 74.1 | 59.8 | 62.5 | 61.2 |
| 200 + mesh | 75.2 | 74.5 | 74.9 | 81.9 | 85.5 | 83.7 |
| CaO | 74.5 | 72.0 | 73.3 | 82.6 | 82.3 | 82.5 |
| | *Wooster silt loam* | | | | | |
| Check | 40.1 | 43.4 | 41.8 | 50.2 | 50.0 | 50.1 |
| 20– 40 mesh | 47.0 | 49.7 | 48.4 | 50.0 | 47.7 | 48.9 |
| 2.5 × 20–40 mesh | 50.4 | 50.2 | 50.3 | 49.3 | 49.1 | 49.2 |
| 60– 80 mesh | 40.5 | 41.5 | 41.0 | 45.2 | 45.5 | 45.4 |
| 100–200 mesh | 49.1 | 50.0 | 49.6 | 42.8 | 43.0 | 42.9 |
| 200 + mesh | 48.8 | 49.3 | 49.1 | 58.7 | 59.4 | 59.1 |
| CaO | 59.4 | 58.3 | 58.9 | 69.3 | 63.4 | 66.4 |
| | *Carrington silt loam* | | | | | |
| Check | 33.8 | 41.0 | 37.4 | 58.1 | 57.4 | 57.8 |
| 20– 40 mesh | 37.3 | 50.0 | 43.7 | 59.9 | 61.6 | 60.3 |
| 2.5 × 20–40 mesh | 57.8 | 27.7 | 42.8 | 60.8 | 61.6 | 61.2 |
| 60– 80 mesh | 38.3 | 47.5 | 42.9 | 53.6 | 53.3 | 53.5 |
| 100–200 mesh | 47.7 | 46.3 | 47.0 | 65.7 | 61.7 | 63.7 |
| 200 + mesh | Lost | Lost | Lost | 53.2 | 60.0 | 61.6 |
| CaO | 50.2 | 50.2 | 50.2 | 66.6 | Lost | 66.6 |

terials the differences resulting from treatment with various grades of limestone are less marked than those noted where soils of coarser texture and lower organic content were employed. However, the evidence points quite definitely to the contention that the finer grades of limestone exert a more beneficial influence upon the process of ammonification than does the coarse material. This is especially significant for the light it throws upon certain pre-

vious results obtained in the ammonification of dried blood and would seem to make the theory advanced in that connection more tenable.

Since in common field practice the amount of limestone applied to the soil is rarely the exact equivalent of the lime-requirements as determined by the

TABLE 17

*The effect of fineness of division of pulverized limestone in varying amounts and* CaO *on ammonification in Norfolk sandy loam. Cottonseed meal*

| TREATMENT | NITROGEN | NITROGEN | AVERAGE NITROGEN |
|---|---|---|---|
| *One-third Veitch lime-requirement applied* | | | |
| | *mgm.* | *mgm.* | *mgm.* |
| Check | 50.0 | 51.6 | 50.8 |
| 0.09 gram, 20– 40 mesh | 56.5 | 54.7 | 55.6 |
| 0.09 gram, 60– 80 mesh | 56.1 | 59.2 | 57.7 |
| 0.09 gram, 100–200 mesh | 61.6 | 61.7 | 61.1 |
| 0.09 gram, 200 + mesh | 60.5 | 60.6 | 60.6 |
| 0.05 gram, CaO | 54.2 | 54.5 | 54.4 |
| *Two-thirds Veitch lime-requirement applied* | | | |
| Check | 50.0 | 51.6 | 50.8 |
| 0.18 gram, 20– 40 mesh | 54.1 | 54.9 | 54.4 |
| 0.18 gram, 60– 80 mesh | 52.8 | 53.8 | 53.3 |
| 0.18 gram, 100–200 mesh | 56.8 | 52.9 | 54.9 |
| 0.18 gram, 200 + mesh | 56.3 | 55.9 | 56.1 |
| 0.10 gram, CaO | 65.3 | 63.1 | 64.2 |
| *Total Veitch lime-requirement applied* | | | |
| Check | 50.0 | 51.6 | 50.8 |
| 0.28 gram, 20– 40 mesh | 51.5 | 50.9 | 51.2 |
| 0.28 gram, 60– 80 mesh | 55.3 | 54.9 | 55.1 |
| 0.28 gram, 100–200 mesh | 59.2 | 59.8 | 59.5 |
| 0.28 gram, 200 + mesh | 62.8 | 63.1 | 63.0 |
| 0.15 gram, CaO | 65.2 | 65.2 | 65.2 |
| *One and one-third Veitch lime-requirement applied* | | | |
| Check | 50.0 | 51.6 | 50.8 |
| 0.37 gram, 20– 40 mesh | 57.7 | 59.0 | 58.4 |
| 0.37 gram, 60– 80 mesh | 63.6 | 63.6 | 63.6 |
| 0.37 gram, 100–200 mesh | 58.8 | 58.6 | 58.7 |
| 0.37 gram, 200 + mesh | 65.8 | 69.0 | 67.4 |
| 0.20 gram, CaO | 68.0 | 69.7 | 68.9 |

Veitch method, the following experiment was performed. Limestone of different grades of fineness and burnt lime were applied to Norfolk sandy loam at the rate of one-third, two-thirds, total and one and one-third of the Veitch lime-requirement of 2300 pounds of CaO per acre. One series received 155

mgm. of nitrogen in the form of cottonseed meal, the other received dried blood. The soils were made up to the optimum moisture content (13 per cent) and incubated for seven days, the ammonia being determined by the magnesian oxide method at the end of that time. The results are presented in tables 17 and 18.

TABLE 18

*The effect of fineness of division of pulverized limestone in varying amounts and* CaO *on ammonification in Norfolk sandy loam (dried blood being used)*

| TREATMENT | NITROGEN | NITROGEN | AVERAGE NITROGEN |
|---|---|---|---|
| *One-third Veitch lime-requirement applied* | | | |
| | *mgm.* | *mgm.* | *mgm.* |
| Check | 70.6 | 69.8 | 70.2 |
| 0.09 gram, 20– 40 mesh | 71.6 | 71.6 | 71.6 |
| 0.09 gram, 60– 80 mesh | 69.5 | 69.6 | 69.6 |
| 0.09 gram, 100–200 mesh | 65.4 | 67.0 | 66.2 |
| 0.09 gram, 200 + mesh | 59.5 | 60.7 | 66.2 |
| 0.05 gram, CaO | 67.7 | 67.4 | 67.6 |
| *Two-thirds Veitch lime-requirement applied* | | | |
| 0.18 gram, 20– 40 mesh | 67.3 | 66.5 | 66.9 |
| 0.18 gram, 60– 80 mesh | 64.2 | 65.0 | 64.6 |
| 0.18 gram, 100–200 mesh | 60.7 | 60.2 | 60.5 |
| 0.18 gram, 200 + mesh | 58.4 | 59.5 | 59.0 |
| 0.10 gram, CaO | 61.1 | 59.0 | 60.1 |
| *Total Veitch lime-requirement applied* | | | |
| 0.28 gram, 20– 40 mesh | 64.9 | 65.8 | 65.4 |
| 0.28 gram, 60– 80 mesh | 64.3 | 62.2 | 63.3 |
| 0.26 gram, 100–200 mesh | 59.5 | 59.1 | 59.3 |
| 0.26 gram, 200 + mesh | 60.1 | 60.2 | 60.2 |
| 0.15 gram, CaO | 59.3 | 59.9 | 59.6 |
| *One and one-third Veitch lime-requirement applied* | | | |
| 0.37 gram, 20– 40 mesh | 67.9 | 70.1 | 69.5 |
| 0.37 gram, 60– 80 mesh | 66.3 | 64.7 | 65.5 |
| 0.37 gram, 100–200 mesh | 59.1 | 61.1 | 60.1 |
| 0.37 gram, 200 + mesh | 60.6 | 59.3 | 60.0 |
| 0.20 gram, CaO | 63.2 | 63.7 | 63.5 |

It will be seen that with but a few exceptions in the cottonseed meal series, an increase in fineness of division of pulverized limestone is responsible for an increased ammonification. This phenomenon appeared regardless of the size of application of limestone. An increase in the quantity of burnt lime caused a still further increase in ammonification. In the dried blood series an increase in fineness of division of limestone caused a corresponding division

in ammonia found. However, as previously interpreted, this may be considered as virtually representing an increase in ammonification accompanied by an increase in assimilation with an increase in fineness of division of pulverized limestone. Again the phenomenon remains constant, regardless of the rate of application of limestone. In general, then, it appears that ammonification as influenced by the different grades of pulverized limestone is independent of the size of application.

Summarizing the results obtained in the present experiments, the following points are worthy of note.

1. An increase in fineness of division of pulverized limestone was responsible for an increase in the ammonification of peptone in solution in Norfolk sandy loam and Penn clay loam, and of cottonseed meal and dried blood in Sassafras sandy loam, which may be correlated with the results obtained in the rate of neutralization of acidity. That is, in the first two weeks of incubation of soil with limestone the differences between treatments are decidedly more marked than in the following weeks when there is a general equalizing tendency manifest.
2. An increase in fineness of division of pulverized limestone is responsible for an increase in nitrification and nitrogen-fixation in solution and fresh soil, which may also be correlated with the rate of ammonification.
3. The ammonification of dried blood and cottonseed meal followed the above tendency in the heavy soils rich in organic matter as well as the lighter soils used.
4. These results of ammonification remain constant regardless of the size of the application of limestone (as determined by the Veitch method).

## THE INFLUENCE OF FINENESS OF DIVISION OF PULVERIZED LIMESTONE ON LOSS BY DRAINAGE[6]

So far as the writer has been able to determine, the recent publication from the Maryland Agricultural Experiment Station (12) represents the sole work on the solubility and loss of different degrees of pulverized limestone when applied to the soil. In the experiments performed by Broughton, Williams, and Frazer (10), both limestone and oyster shells of varying degrees of fineness were employed. The solubility of these samples which had been subjected to mechanical analysis was determined in water, and water charged with carbon dioxide at 3°, 12°, 14°, and 23°C., representing the temperatures of winter, spring, autumn, and summer, respectively. It was found that with an increase in fineness of division of pulverized limestone there was an increase in solubility, the same phenomenon being observed with an increase in temperature. The solubility and the different materials were then determined

[6] The writer wishes to express appreciation for his assistance to Mr. R. C. Cook, with whom this experiment was begun. Owing to unforeseen circumstances the latter was unable to continue this work.

by analyzing the soil solution obtained from large pots treated with lime. Unfortunately, no duplicate treatments are mentioned, and since the soils considered as checks received applications of either CaO or $CaCO_3$, the experiment is open to criticism on these grounds. This is further emphasized by the fact that the differences between treatments were slight. However, it was shown that the solubility of these materials increased with an increase in the fineness of division of limestone. In other words the greater the surface of limestone exposed, the greater was its solubility. Two definite conclusions were recorded from interesting data of field experiments still in progress. The authors maintain that in order to furnish as much calcium as is present in burnt lime, limestone should be ground so that 90 per cent passes an 80-mesh sieve. Their conclusion is corroborated by the work reported in this thesis.

There is a considerable amount of data concerning the distribution, changes and loss of limestone by leaching which has been reported by Blanck (7), Broughton (9), Creydt, Von Seelhorst and Wiems (16), Frear (30), Hilgard (40), Johnson (49), Lyon and Bizzell (69), MacIntyre, et al. (72), Miller (74), and Veitch (109). The effect of adding limestone in excess of the amount required to neutralize acidity has been studied by Gardner and Brown (34), White (107), and Veitch (109).

In order to study the influence of the fineness of pulverized limestone on the loss of lime in drainage, the following method was devised and put into operation in 1914.[7]

Large galvanized iron pots, 16 inches in diameter and 12 inches deep, standing on three legs, were fitted with stopcocks to permit drainage. The bottom of the pot had a slight depression in which were placed large pebbles. The pots were fitted with a pair of handles rivetted to the sides, and the whole painted to prevent rusting. A layer of fine pebbles was placed in the bottom of the pot, and over this was placed 120 pounds of Sassafras sandy loam which had been sieved through an 8-mesh screen (plate 1). A lever arrangement with grippers for the handles of the pot, and a counterpoise was devised for keeping the pots at constant weight. Two series of new pots were placed upon a greenhouse table. A hole was drilled under each stopcock to permit the fastening of a rubber tube which extended down into a bottle to collect the drainage water. A board support was nailed close to the ground upon which the bottles might be placed in proper position. After the harvesting of each crop, the drainage water was collected by flushing through enough water to obtain 3800 cc. of drainage in each collecting bottle. This method is, of course, open to objection, in that continuous percolation would give a more adequate representation of the drainage water, but the greenhouse conditions were such as to make such a procedure impracticable.

[7] Broughton (10) and the United States Bureau of Plant Industry, at Washington, D. C., have since described similar apparatus for the same purpose (122).

The experiment was continued over a period of time which permitted the growth of several different crops, the soil being treated with pulverized limestone of different grades in quantity sufficient to fulfill the lime-requirement as determined by the Veitch method. One series of pots received ammonium sulfate at the rate of 600 pounds to the acre, while the other series received no addition of nitrogen. The check pots received minerals above, while the others received minerals in addition to the special treatment. Before planting the first crop the soil from each pot was removed to a depth of $6\frac{2}{3}$ inches, while 15 grams of acid phosphate (14 per cent $P_2O_5$) and 7.5 grams of muriate of potash (50 per cent $K_2O$) were thoroughly incorporated together with 57 grams of pulverized limestone of different grades of fineness. The soil, was designated as Sassafras sandy loam which had been unlimed, and unfertilized

TABLE 19

*The effect of fineness of division of pulverized limestone on the yield of barley grown on Sassafras sandy loam in drainage tanks with and without the addition of nitrogen*

| TREATMENT | WEIGHT OF CROP | DUPLICATE | AVERAGE | NITROGEN | NITROGEN | AVERAGE NITROGEN | TOTAL NITROGEN |
|---|---|---|---|---|---|---|---|
| *Without the addition of nitrogen* | | | | | | | |
| | *grams* | *grams* | *grams* | *per cent* | *per cent* | *per cent* | *grams* |
| Check..... | 45.5 | 36.5 | 41.0 | 1.18 | 0.89 | 1.04 | 0.43 |
| 20– 40 mesh... | 79.0 | 80.5 | 79.7 | 0.83 | 0.96 | 0.90 | 0.71 |
| 60– 80 mesh... | 95.0 | 88.5 | 91.7 | 0.86 | 0.86 | 0.86 | 0.79 |
| 100–200 mesh... | 93.0 | 91.5 | 92.2 | 0.88 | 0.84 | 0.86 | 0.79 |
| 200 + mesh.... | 81.5 | 85.0 | 83.2 | 0.82 | 0.84 | 0.83 | 0.69 |
| *12 grams* $(NH_4)_2SO_4$ *applied* | | | | | | | |
| Check..... | 48.0 | 55.5 | 51.7 | 1.30 | 1.32 | 1.31 | 0.68 |
| 20– 40 mesh... | 82.0 | Lost | 82.0 | 1.28 | 1.33 | 1.31 | 1.07 |
| 60– 80 mesh... | 87.0 | 73.0 | 80.0 | 1.33 | 1.08 | 1.21 | 0.97 |
| 100–200 mesh... | 74.0 | 92.0 | 83.0 | 1.33 | 1.22 | 1.28 | 1.06 |
| 200 + mesh.... | 78.0 | 67.5 | 72.7 | 1.32 | Lost | 1.32 | 0.96 |

and had a lime-requirement (Veitch) of 100 pounds CaO per acre. The pots were maintained at optimum moisture content. Seven grams of ammonium sulfate were applied to each pot of one series one week before planting 100 seeds of barley per pot. The other 5 grams were added after the seedlings had been thinned to 50 plants.

The results of yield and nitrogen content of the first crop grown in these tanks is presented in table 19.

Some discrepancies which appear are due to attacks from fungi which did not invalidate the general tendency exhibited, namely, with an increase in the fineness of division of pulverized limestone up to the 200-mesh product there was an increase in crop yield and total nitrogen in grams. This is to be seen both in the series receiving no nitrogen and that receiving 12 grams of

ammonium sulfate per pot. From the figures recorded in the columns marked "average per cent of nitrogen" it is apparent that the differences as a result of variation in treatment are so slight as to be negligible. Thus the total nitrogen expressed in grams (last column) varies almost as the crop yield. The question which is immediately suggested by these results is: why are the yields obtained with the finest limestone smaller than those where the coarser material was used? The explanation which appears most plausible is that in such a light open sandy soil the finer material is washed down below the root zone of the plant, therefore it does not neutralize the soil acidity or increase the available nitrogen supply of the plant to the same extent as does the coarser material which is actually less efficient. This hypothesis is strengthened by certain subsequent results concerned with the planting of alfalfa (which is one of the deepest-rooted of all crops) which seems to respond with a greater yield to the finer limestone. Likewise, the fact that more lime is lost in leaching from the soils receiving the finer material, as will be shown presently, also may be considered as corroborative evidence. A further consideration may be advanced, namely, it is possible that the finer material, being more active, effects a reversion of the acid phosphate sufficient to limit plant growth to some degree. However, in view of the large amount of acid phosphate applied, this does not appear tenable. Again the nitrogen in the soil may have been oxidized to nitrates more rapidly with soils receiving the finer limestone, and rapidly transformed into insoluble nitrogenous compounds before the plant could use them. As has been previously stated, after harvesting the crops (which had been allowed to grow to maturity), water was flushed through the soil and the 3800 cc. of drainage water collected. Duplicate aliquot samples of 5 cc. were taken for the determination of ammonia by the Nessler colorimetric method (98), and 25-cc. portions were taken for nitrate nitrogen determinations according to the phenoldisulphonic acid method. The 3740 cc. of drainage water were then evaporated to about 400 cc. After 10 cc. of hydrochloric acid (concentrated) were added, the solution was made up to 500 cc. and kept in a tightly-stoppered Jena flask until used for the determination of calcium by the standard permanganate titration method.

From the results recorded in table 20, it will be noted that in both series an increase in fineness of division of pulverized limestone was accompanied by a decrease in loss of ammonia in the drainage water. As was to be expected the series receiving ammonium sulfate lost more ammonia than the series receiving no nitrogen. The determination of nitrates and calcium in the drainage waters led to no definite conclusion because of lack in agreement between duplicate treatments, therefore the results were not tabulated.

After permitting the soil to dry out sufficiently to restore the optimum moisture condition, buckwheat was planted and the crop grown to maturity. In this case, no further applications of limestone were made, but the usual 12 grams of ammonium sulfate were applied to each pot of the series receiving nitrogen. The yield and nitrogen content of the crops are recorded in table 21.

In the series receiving no nitrogen there is an increase in crop yield with 60 to 80-mesh as compared with 20 to 40-mesh limestone. The material finer than 60-mesh caused a decline in yield which, however, remained superior

TABLE 20

*Analysis of first drainage water for ammonia*

| TREATMENT | $NH_3$ IN 3800 CENTIMETERS | DUPLICATE $NH_3$ | AVERAGE $NH_3$ | DECREASE OVER CHECK $NH_3$ |
|---|---|---|---|---|
| *No nitrogen applied* | | | | |
| | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| Check | 0.532 | 0.760 | 0.646 | |
| 20– 40 mesh | 0.026 | 0.026 | 0.026 | 0.619 |
| 60– 80 mesh | 0.030 | 0.019 | 0.025 | 0.621 |
| 100–200 mesh | 0.011 | 0.019 | 0.015 | 0.630 |
| 200 + mesh | 0.019 | 0.000 | 0.010 | 0.636 |
| *12 grams* $(NH_4)_2SO_4$ *applied* | | | | |
| Check | 1.140 | 1.133 | 1.235 | |
| 20– 40 mesh | 0.570 | 0.950 | 0.760 | 0.475 |
| 60– 80 mesh | 0.836 | 0.608 | 0.722 | 0.513 |
| 100–200 mesh | 0.570 | 0.228 | 0.399 | 0.836 |
| 200 + mesh | 0.285 | Lost | 0.285 | 0.950 |

TABLE 21

*The effect of fineness of division of pulverized limestone on the yield of buckwheat in drainage tanks with and without applications of nitrogen*

| TREATMENT | WEIGHT OF CROP | DUPLICATES | AVERAGE | NITROGEN | NITROGEN | AVERAGE NITROGEN | TOTAL NITROGEN |
|---|---|---|---|---|---|---|---|
| *Without nitrogen* | | | | | | | |
| | *grams* | *grams* | *grams* | *per cent* | *per cent* | *per cent* | *grams* |
| Check | 38.0 | 15.7 | 26.9 | 1.99 | 1.95 | 1.97 | 0.53 |
| 20– 40 mesh | 44.9 | 43.0 | 44.0 | 1.31 | 1.43 | 1.37 | 0.60 |
| 60– 80 mesh | 68.7 | 71.7 | 70.2 | 1.17 | 1.33 | 1.25 | 0.88 |
| 100–200 mesh | 58.0 | 53.0 | 55.5 | 1.22 | 1.29 | 1.26 | 0.70 |
| 200 + mesh | 54.8 | 50.7 | 52.8 | 1.56 | 1.53 | 1.55 | 0.82 |
| *12 grams* $(NH_4)_2SO_4$ *applied* | | | | | | | |
| Check | 16.0 | 17.6 | 16.8 | 2.37 | 2.37 | 2.37 | 0.40 |
| 20– 40 mesh | 80.4 | 88.6 | 84.5 | 1.96 | 2.36 | 2.16 | 1.82 |
| 60– 80 mesh | 93.5 | 103.4 | 99.0 | 1.86 | 2.14 | 2.05 | 2.03 |
| 100–200 mesh | 90.9 | 84.0 | 87.5 | 2.20 | 2.14 | 2.17 | 1.90 |
| 200 + mesh | 88.5 | 94.9 | 92.7 | 2.12 | 2.12 | 2.12 | 1.97 |

to the 20-mesh limestone. There was very little variation in the percentage of nitrogen, although in this instance the 200-mesh limestone gave a considerably higher percentage than did the other grades of limestone. The reason

for the high nitrogen content in the checks is, of course, the relatively low yield, this phenomenon being of too common occurrence to require further comment. Again in general, the total nitrogen in grams is roughly proportional to the yields obtained. In the series receiving ammonium sulfate it is at once evident that the total yield of all pots (with the exception of the checks, due to higher acidity), is higher than in the case of the soils receiving no nitrogen. Again, the results with the different grades of limestone and ammonium sulfate exactly parallel those obtained where nitrogen was not added. The variations in nitrogen content are negligible. The fact that the 60-mesh gives a superior yield to that obtained with the finer limestone has already been discussed in connection with the previous crop. It is especially interesting to note that the yields where fine pulverized limestone is

TABLE 22

*Neutralization of acidity by different degrees of fineness of pulverized limestone (Sassafras sandy loam after growing buckwheat)*

| TREATMENT | CaO PER ACRE | CaO PER ACRE | AVERAGE CaO PER ACRE |
|---|---|---|---|
| *No nitrogen applied* | | | |
| | *pounds* | *pounds* | *pounds* |
| Check | 4,900 | 4,600 | 4,750 |
| 20– 40 mesh | 3,100 | 3,100 | 3,100 |
| 60– 80 mesh | 2,600 | 1,600 | 2,100 |
| 100–200 mesh | 1,750 | 1,650 | 1,700 |
| 200 + mesh | 1,650 | 1,650 | 1,650 |
| *12 grams* $(NH_4)_2SO_4$ *applied* | | | |
| Check | 5,200 | 4,500 | 4,850 |
| 20– 40 mesh | 3,600 | 3,400 | 3,500 |
| 60– 80 mesh | 3,600 | 2,500 | 3,050 |
| 100–200 mesh | 2,250 | 3,300 | 2,375 |
| 200 + mesh | 1,900 | 2,000 | 1,950 |

used without any addition of nitrogen are only slightly inferior to the yields obtained where coarse limestone was accompanied by an application of ammonium sulfate at the rate of 660 pounds per acre.

In order to cast further light upon the neutralization of soil acidity by the different grades of pulverized limestone, the lime-requirement of all soils was determined after harvesting the crops and before leaching. This was done by taking three cores of soil the entire depth of the pot, mixing them thoroughly and measuring out a representative sample of 200 grams, of which 10-gram portions were used for the Veitch determinations. The results obtained are shown in table 22 and may be considered of primary importance.

In the first place it is evident in both series that an increase in fineness of division is responsible for a diminution of the lime-requirement. Again,

it will be seen that in comparing the series receiving ammonium sulfate with the series receiving no nitrogen, the former had a uniformly higher lime-requirement than the latter, regardless of the grades of limestone used. While the agreement between duplicates is not in all cases what might be desired, nevertheless, the general trend is unmistakable. This, after a fashion, may be considered as bearing upon the discussion of crop yield, namely, the composite sample of soil receiving 200-mesh limestone has a lower lime-requirement than where the coarser material was used, but the fact that the plants could not take advantage of this indicates that much of the neutralization took place below the root zone. Consequently, the yields obtained with medium fine material are superior to those obtained with very fine limestone.

After harvesting the crops, the soil in each pot was leached as previously described and duplicate samples of 20 cc. were taken for the determination of acidity by titration with N/50 NaOH, phenolphthalein being used; and of alkalinity by titration with N/50 HCl, with methyl orange as an indicator. The general tendency was for a decrease in acidity with an increase in fineness of division of pulverized limestone, and for an increase in alkalinity. The series receiving nitrogen showed a higher acidity than where ammonium sulfate was applied. Thus it required 0.86 cc. of N/50 NaOH to neutralize the acidity where 200-mesh was used, as against 1.75 cc. where 20-mesh limestone was used in the series receiving no nitrogen, and 1.46 cc. as against 1.96 cc. in the series receiving ammonium sulfate. The other results were not tabulated because of lack of sufficient agreement between duplicates. This is likewise true of the determinations of ammonia, nitrates and calcium, although the general tendency exhibited was that an increase in fineness of division of pulverized limestone was responsible for a decrease in the loss of ammonia and nitrate nitrogen and an increase in the loss of calcium in the drainage waters.

The next crop to be grown in these pots was rape. This was selected because of the fact that the experiment in progress required that there be no addition of nitrogen to one series of soils, and rape is not regarded as a heavy feeder on nitrogen. Of course, the root residues of former crops furnished a source of nitrifiable material and this is undoubtedly the principal reason for the good yields obtained. Fifteen grams of acid phosphate and 7.5 grams of muriate of potash were added at the outset of the experiment and limestone was added to all soils in excess of the lime-requirement as determined after harvesting buckwheat (table 22). These applications were made on the basis of two-thirds in excess of the lime-requirement of the soils having the greatest acidity (the checks receiving one-fourth in excess in the form of 200-mesh limestone). The additions of fertilizer and lime were made two weeks before planting 20 rape seeds. The seedlings were thinned to 10 in number, and the additional application of 5 grams of ammonium sulfate made. The crop was grown to maturity but difficulty was experienced in combatting cabbage worms. This pest was finally exterminated, after creating considerable dam-

age, by sprinkling the leaves with powdered lead arsenate. The results of crop yield and nitrogen content are recorded in table 23.

While the differences between treatments are not pronounced, due to the fact that an excess of lime was applied, nevertheless, it is evident in both series that the 60 to 80-mesh limestone yielded superior results while the 200-mesh appeared to better advantage than did the 20-mesh limestone, which corroborates the conclusions based on the yields with the previous two crops. Again the percentage of nitrogen in both series is highest with the 200-mesh limestone, while the total nitrogen in grams parallels the crop yields. It will also be seen the series receiving ammoinum sulfate gave higher yields and nitrogen content than the series receiving no nitrogen. However, the yields

TABLE 23

*The effect of fineness of division of pulverized limestone on the yield of rape in drainage tanks (two-thirds in excess of lime-requirement)*

| TREATMENT | WEIGHT OF CROP | DUPLICATES | AVERAGE | NITROGEN | NITROGEN | AVERAGE NITROGEN | TOTAL NITROGEN |
|---|---|---|---|---|---|---|---|
| | | | *No nitrogen applied* | | | | |
| | *grams* | *grams* | *grams* | *per cent* | *per cent* | *per cent* | *grams* |
| Check..... | 55.4 | 68.4 | 61.9 | 1.50 | 1.55 | 1.53 | 0.95 |
| 20– 40 mesh... | 56.0 | 60.0 | 58.0 | 1.31 | 1.29 | 1.30 | 0.75 |
| 60– 80 mesh... | 71.0 | 72.5 | 71.8 | 1.29 | 1.31 | 1.30 | 0.93 |
| 100–200 mesh... | 72.2 | 64.2 | 68.2 | 1.28 | 1.40 | 1.34 | 0.91 |
| 200 + mesh.... | 66.2 | 69.2 | 67.7 | 1.42 | 1.35 | 1.39 | 0.94 |
| | | | *12 grams* $(NH_4)_2SO_4$ *applied* | | | | |
| Check..... | 78.7 | 70.5 | 74.6 | 2.63 | 2.85 | 2.79 | 2.08 |
| 20– 40 mesh... | 77.7 | 72.0 | 74.9 | 1.89 | 1.81 | 1.85 | 1.39 |
| 60– 80 mesh... | 95.0 | 81.2 | 88.1 | 1.81 | 1.87 | 1.84 | 1.62 |
| 100–200 mesh... | 76.7 | 71.0 | 73.9 | 1.88 | 1.88 | 1.88 | 1.39 |
| 200 + mesh.... | 76.0 | 76.6 | 76.3 | 1.88 | 1.92 | 1.90 | 1.45 |

with the finer limestone without nitrogen were only slightly inferior to those obtained with the coarse limestone where ammonium sulfate had been added at the rate of 600 pounds to the acre. Thus one might argue that limestone was responsible for an increased supply of available nitrogen to the plant.

Following the previous procedure of determining the lime-requirement of all the soils before leaching, the results are presented in table 24.

It will be seen that in both series an increase in fineness of division of pulverized limestone was accompanied by a decrease in the lime-requirement. There is mutual corroboration in the fact that the check soils, as well as those regularly receiving 200-mesh limestone, were alkaline, for it will be remembered that the former received an excessive application of 200-mesh limestone. It is further interesting to note that the acidity developed in the soils receiving

60-mesh is only one-half (or less) of that developed in the soils receiving 20-mesh limestone.

The soils were leached in the usual manner and the acidity and alkalinity titrated as before. Again, the results indicate that with an increase in fineness of division of pulverized limestone there is a decrease in acidity and an increase in alkalinity. For example, in the series receiving no nitrogen, 20 cc. of drainage water from the soil treated with 20-mesh requires 3.28 cc. of N/50 NaOH to effect neutralization while 200-mesh requires only 2.53 cc. In the series receiving ammonium sulfate, the former treatment requires 3.93 cc. and the latter only 1.80 cc. The results of the ammonia and nitrate determinations were too variable to permit of definite conclusions, although there

TABLE 24

*Neutralization of acidity by different degrees of fineness of division of pulverized limestone (Sassafras sandy loam after growing rape). Two-thirds in excess of lime requirement applied—200-mesh limestone applied to check*

| TREATMENT | CaO | CaO | AVERAGE CaO |
|---|---|---|---|
| *No nitrogen applied* | | | |
| | *pounds* | *pounds* | *pounds* |
| Check | Alkaline | Alkaline | Alkaline |
| 20– 40 mesh | 2,500 | 2,300 | 2,400 |
| 60– 80 mesh | 1,300 | 1,200 | 1,250 |
| 100–200 mesh | Alkaline | Alkaline | Alkaline |
| 200 + mesh | Alkaline | Alkaline | Alkaline |
| *12 grams* $(NH_4)_2SO_4$ *applied* | | | |
| Check | 100 | Alkaline | 50 |
| 20– 40 mesh | 3,500 | 2,900 | 3,200 |
| 60– 80 mesh | 1,900 | 800 | 1,350 |
| 100–200 mesh | 100 | Alkaline | 50 |
| 200 + mesh | Alkaline | Alkaline | Alkaline |

appeared to be a tendency for an increase in fineness of division of pulverized limestone to cause a decrease in loss of ammonia and nitrates. Thus in the series receiving ammonium sulfate the 20-mesh treated soils contained 2.10 mgm. of nitrogen nitrates, while the soils treated with 200-mesh yielded only 0.46 mgm. of nitrogen. Again in the series receiving no nitrogen, with the 20-mesh there were 1.653 mgm. of nitrogen in the form of ammonia in the drainage compared with 0.798 mgm. of nitrogen in the case of the 200-mesh.

The results of the calcium determinations in the drainage water are presented in table 25.

It will be seen that in both series (with one exception) there is an increased loss of lime with an increase in fineness of division of pulverized limestone.

While the duplicates are not always in close agreement, nevertheless, the general tendency of the averages is pronounced.

Since rape grew so well under the conditions of its first trial, it was deemed advisable to use the same plant again. No lime or fertilizer was added other than the ammonium sulfate in the manner previously described. Twenty-five seeds were planted in each pot, and these were later thinned to 15 seedlings. An inspection of the results in table 26 shows slight differences as a result of treatment.

However, as usual the 60-mesh limestone is superior to the other grades. The yields on the soils receiving ammonium sulfate are distinctly superior to those receiving no nitrogen. Because of the nature of these results, differences in drainage waters were not to be anticipated, and consequently the soils were not leached.

TABLE 25

*Analysis of drainage water: determination of calcium. Third series*

| TREATMENT | Ca | DUPLICATES | AVERAGE |
|---|---|---|---|
| *No nitrogen applied* | | | |
| | *mgm.* | *mgm.* | *mgm.* |
| Check | 78.3 | 73.0 | 75.7 |
| 20– 40 mesh | 77.9 | Lost | 77.9 |
| 60– 80 mesh | 71.7 | 70.0 | 70.9 |
| 100–200 mesh | 81.8 | 80.5 | 81.2 |
| 200 + mesh | 85.4 | 89.8 | 87.6 |
| *12 grams* $(NH_4)_2SO_4$ *applied* | | | |
| Check | 51.9 | 79.2 | 65.6 |
| 20– 40 mesh | 69.5 | 112.2 | 90.9 |
| 60– 80 mesh | 153.1 | 49.3 | 101.2 |
| 100–200 mesh | 176.0 | Lost | 118.4 |
| 200 + mesh | 176.0 | 92.0 | 134.0 |

Summarizing the results obtained in the work just discussed concerning the growth of various crops in large pots permitting the collection of drainage water and the analyses thereof, the following points have been established, a light sandy loam (Sassafras) being treated with different degrees of fineness of pulverized limestone, which had been cropped to barley, buckwheat, and rape (twice).

1. Both in the series receiving an application of ammonium sulfate at the rate of 660 pounds to the acre and that receiving no nitrogen, the 60-mesh limestone gave the highest yields. This is explained by the fact that the finer grades of limestone were washed down below the root zone where they became less effective in neutralizing soil acidity and making plant-food available. The 200-mesh gave higher yields than 20-mesh limestone.

2. The highest average per cent of nitrogen in the various crops was obtained with the 200-mesh limestone in the series with and without nitrogen.

3. The soils receiving an application of ammonium sulfate at the rate of 600 pounds to the acre yielded crops superior to those which received no nitrogen, when the limestone treatment remained the same.

4. The best yields with the finer limestone with and without nitrogen are only slightly inferior to the yields obtained with the coarsest limestone on soil receiving 660 pounds of ammonium sulfate to the acre. This suggests a possible value for finely-pulverized limestone, namely, the making available of nitrogen in the soil.

TABLE 26

*The effect of fineness of division of pulverized limestone on the second successive crop of rape grown in drainage pots*

| TREATMENT | WEIGHT OF CROP | DUPLICATES | AVERAGE |
|---|---|---|---|
| *Without nitrogen* | | | |
| | *grams* | *grams* | *grams* |
| Check | 17.7 | 20.9 | 19.3 |
| 20– 40 mesh | 19.8 | 21.5 | 20.7 |
| 60– 80 mesh | 24.2 | 23.6 | 23.9 |
| 100–200 mesh | 20.8 | 17.8 | 19.3 |
| 200 + mesh | 18.0 | 23.1 | 20.6 |
| *12 grams* $(NH_4)_2SO_4$ *applied* | | | |
| Check | 29.1 | 10.3* | 29.1 |
| 20– 40 mesh | 32.2 | 32.2 | 32.2 |
| 60– 80 mesh | 33.0 | 32.1 | 32.6 |
| 100–200 mesh | 31.5 | 31.5 | 31.5 |
| 200 + mesh | 25.3* | 31.6 | 31.6 |

* Poor growth observed.

5. Large galvanized iron pots were devised for growing crops in the greenhouse and collecting drainage water. The analyses of the latter indicated that an increase in fineness of division of pulverized limestone was responsible for a decreased loss of ammonia and nitrate nitrogen, thereby conserving the nitrogen supply. There was an increased loss of calcium, however, which is an important consideration from the standpoint of economy in the application of lime.

6. As in the preceding experiments an increase in fineness of division of pulverized limestone was responsible for a corresponding decrease in the lime-requirement of the soil. The titration of the acidity and alkalinity of the drainage waters indicated that an increase in fineness of division of pulverized limestone was accompanied by a decrease in acidity as measured by N/10 NaOH and an increase in alkalinity as measured by N/10 HCl.

## SUMMARY

Considering as a whole the results set forth in this investigation, some interesting facts have come to light which appear to have a direct bearing upon the agricultural practice of liming. The influence of fineness of division of pulverized limestone, upon crop yield on various soils has been discussed, together with the effect upon the chemical and bacteriological factors of the soil. The more important points which have been established under the limitations of the experiment may be summarized as follows.

1. An increase in fineness of division of pulverized limestone from 20 to 40, 60 to 80, 100 to 200, to finer than 200-mesh is responsible for a proportional increase in the yield and total nitrogen content of crimson clover on several soil types (representing extensive fertile areas in the United States); and a corresponding decrease in lime-requirement.

2. From the above standpoint there was little choice between burnt lime and 200-mesh limestone.

3. Employing an apparatus devised for measuring the rate of neutralization of soil acidity by different grades of pulverized limestone, it was found that in three different soils the limestone finer than 60-mesh required about three weeks, while 20-mesh limestone required more than seven weeks to effect neutralization. An increase in the quantity of limestone required for neutralization was proportional to an increase in fineness of division of pulverized limestone, for any given period of time.

4. An increase in fineness of division of pulverized limestone is responsible for an increase in the activity of the bacteriological processes of ammonification, nitrification and nitrogen fixation (within certain limitations) as measured in soil and solution. This holds true regardless of whether the amount of limestone applied is less or somewhat more than the indicated Veitch lime-requirement.

5. The increase in bacteriological activities with an increase in fineness of division of pulverized limestone may be correlated directly with the chemical factors involved, i.e., the rate of neutralization of acidity, etc., and also crop yield.

6. With pots cropped to barley, buckwheat and rape (twice) which had been designed to permit the collection of drainage water, it was found that on a light open sandy loam the highest yields were obtained from soils treated with 60 to 80-mesh limestone. Two hundred-mesh proved superior to 20-mesh limestone but inferior to the former, because the material was probably washed down below the root zone. These results were paralleled both in the series with and without nitrogen, although the yields in the former case were superior to those in the latter. However, the fine limestone without nitrogen gave almost as high a yield in several instances as the coarse material with an application of 660 pounds of ammonium sulfate to the acre. The highest average per cent of nitrogen, however, was obtained with 200-mesh limestone.

7. An increase in the fineness of division of pulverized limestone was accompanied by a reduction in the lime-requirement. An analysis of the drainage waters indicated that there was a decreased loss of ammonia and nitrate nitrogen but an increased loss of calcium.

8. In general, an increase in the fineness of division of pulverized limestone is responsible for a proportional increase in crop yield, as well as for exerting a beneficial influence on the chemical factors in the soil. Furthermore, 200-mesh limestone may be regarded as effective as burnt lime.

In conclusion, it is a privilege to express appreciation to Dr. J. G. Lipman for his valuable advice so generously rendered in the planning and execution of this investigation. The writer is also indebted to Professor A. W. Blair for his assistance and interest throughout the work; and to his colleagues in the laboratory for many timely suggestions. Thanks are due Dr. H. E. Kiefer, Mr. E. C. Carhart and Mr. E. Meyer for material furnished by them.

## LITERATURE CITED

(1) Adams, G. E. 1907 A study of Rhode Island soil requirements. R. I. Agr. Exp. Sta. Bul. 121.

(2) Ames, J. W. 1912 Carriers of lime. Ohio Agr. Exp. Sta. Circ. 123.

(3) Ames, J. W., and Schollenberger, C. J. 1917 Composition of limestone. *In* Ohio Agr. Exp. Sta. Monthly Bul., vol. 2, no. 1, p. 15–17.

(4) Baessler, P. 1899 *In* Jahresb. Vers. Sta. Breslau. Cited by Gerlach (35).

(5) Barker, J. F., and Collison 1915 Limestone for acid soils. N. Y. Agr. Exp. Sta. Geneva, Bul. 400.

(6) Bernard, A. 1892 Le Calcaire, p. 59–62. Macon, Protal Freres.

(7) Blanck, E. 1909 The influence of lime on the movement of waters in soils. *In* Landw. Jahrb., Bd. 38, No. 5–6, p. 715–758.

(8) Brehmer 1911 Die Bedeutung einer feiner Mahlung bei Kalkmergeln. *In* Illus. Landw. Ztg., Bd. 31, No. 89, p. 832.

(9) Broughton, L. B. 1912 Distribution and loss from the soil of lime. Md. Agr. Exp. Sta. Bul. 166.

(10) Broughton, L. B., Williams, R. C., and Frazer, G. S. 1916 Tests of the availability of different grades of ground limestone. Md. Agr. Exp. Sta. Bul. 193.

(11) Brown, P. E. 1911 Some bacteriological effects of liming. Iowa Agr. Exp. Sta. Research Bul. 2.

(12) Brown, P. E. 1912 Bacteriological studies of field soils. I. Iowa Agr. Exp. Sta. Research Bul. 5.

(13) Brown, P. E. 1913 Methods for the bacteriological examination of soils. Iowa Agr. Exp. Sta. Research Bul. 11.

(14) Brown, P. E. 1914 Soil acidity and the liming of Iowa soils. Iowa Agr. Exp. Sta. Bul. 151.

(15) Cameron, F. R., and Bell, J. M. 1908 Solubility of carbonates. U. S. Dept. Agr. Bur. Soils, Bul. 49, p. 39.

(16) Creydt, von Seelhorst, and Wiems 1901 Untersuchungen über Drainage-wasser. *In* Jour. Landw., Bd. 49, p. 251–275.

(17) Densch, A. 1913 Zur Frage der Schädlichen Wirkung zu starker Kalkgaben, etc. *In* Landw. Jahrb., Bd. 44, p. 331.

(18) Dumont, J. 1904 Action of calcium compounds on mobilization of potash. *In* Bul. Soc. Nat. Agr. France, t. 64, no. 5, p. 379–384.

(19) ELLETT, W. B. 1910 Lime for Virginia farms. Va. Agr. Exp. Sta. Bul. 187.
(20) ENGBERDING, D. 1909 Vergleichende Untersuchungen über die Bakterien zahl im Ackerboden, etc. *In* Centbl. Bakt. (etc.), Abt. 2, Bd. 23, p. 569–642.
(21) FEILITZEN, H. VON 1910 On the influence of the fineness of lime on its action as an amendment of peat soils low in lime. *In* Svenska Mosskulturför. Tidskr., v. 24, p. 95.
(22) FEILITZEN, H. VON 1915 Der Wert einiger Kalkreicher Abfall produkte im Vergleich mit geloschtem Kalk und Kalksteinmehl. *In* Svenska Mosskulturför. Tidskr., v. 28, no. 3–4, p. 210–218. *Abs. in* Centbl. Agr. Chem., Bd. 44, No. 4–5, p. 160, 161.
(23) FIPPIN, E. O. 1910 The relation of lime to soil improvement. N. Y. Agr. Exp. Sta. Circ. 7.
(24) FISCHER, H. 1909 The influence of lime on bacteria. *In* Landw. Vers. Sta., Bd. 70, No. 5–6, p. 335–342.
(25) FISCHER, H. 1911 Versuche uber Stickstoffumsetzung in verschiedenen Böden. *In* Landw. Jahrb., Bd. 41, p. 775–882.
(26) FITTBOGEN, J. 1874 Über die Veränderungen welche der inorganischer Verbindung enthaltene Stickstoff des Moorbodens unter dem Einflusse verschiedener als Düngungs und Meliorationsmittel, gebrauchlicher Substanzen erfährt. *In* Landw. Jahrb., Bd. 3, p. 109–120.
(27) FRAAS 1861 Fortsetzung der Angaben der Lysimeter. *In* Ergeb. Landw. Chem. Vers. Sta. Bayer. Landw. Ver. Heft 3, p. 79.
(28) FRAPS, G. S. 1908 The production of active nitrogen in the soil. Tex. Agr. Exp. Sta. Bul. 106.
(29) FREAR, W. 1900 The agricultural use of lime. Penn. Agr. Exp. Sta. Ann. Rpt. 1899–1900, p. 15–154.
(30) FREAR, W. 1915 Sour soils and liming. Penn. Dept. Agr. Bul. 261.
(31) FREAR, W., AND THOMAS, W. 1915 The lime-magnesia ratio in soil amendments. *In* Jour. Indus. Engin. Chem., v. 7, no. 12, p. 1042.
(32) FRED, E. B. 1912 A study of nitrification in certain types of Virginia soil. Va. Agr. Exp. Sta. Ann. Rpt. 1911–1912, p. 174–201.
(33) GARDNER, F. D. 1914 The use of lime on land. Penn. Agr. Exp. Sta. Bul. 131.
(34) GARDNER, F. D., AND BROWN, B. E. 1910–1911 The lime-requirement of the general fertilizer plots as determined periodically. Penn. Agr. Exp. Sta. Ann. Rpt. 1910–1911, p. 25–76.
(35) GERLACH 1911 Zur Bewertung der Kalkmergel. *In* Deut. Landw. Presse, Bd. 38, No. 35, p. 409.
(36) GRANTHAM, A. E. 1916 Report of the agronomist. Del. Agr. Exp. Sta. Ann. Rpt. 1915, p. 11.
(37) HALL, A. D., AND MILLER, N. H. J. 1905 The effect of plant growth and of manures upon the retention of bases by the soil. *In* Proc. Roy. Soc. (London), Ser. B, vol. 77, no. B 514, p. 1–32.
(38) HARDT, B. 1905 Über die vierjahrigen Ergebnisse eines Kultur-und vergleichenden Kaldungungsversuches auf Heideboden. *In* Deut. Landw. Presse, Bd. 32, p. 253–254.
(39) HEINRICH, R. 1908 Mergel und Mergeln.
(40) HILGARD, E. W. 1909 Soils: their formation, properties, composition, and relations to climate and plant growth in the humid and arid regions, p. 22. Macmillan Co. New York.
(41) HOFFMAN, M. 1905 Fertilizer experiments with lime. *In* Arb. Deut. Landw. Gesell., 1905, No. 106, p. 276.
(42) HOFFMAN, C., AND HAMMER, B. W. 1910 Some factors concerned in the fixation of nitrogen by Azotobacter. Wis. Agr. Exp. Sta. Research Bul. 12, p. 163–164.

(43) HOLLEMAN, A. F. 1893 Bestimmungen der Löslichkeit sogennanter unlöslicher Salze. *In* Ztschr. Phys. Chem., Bd. 12, p. 125.

(44) HOPKINS, C. G. 1903 Soil treatment for wheat in rotations with special reference to southern Illinois soils. Ill. Agr. Exp. Sta. Bul. 88.

(45) HOPKINS, C. G. 1905 Soil treatment for the lower Illinois glaciation. Ill. Agr. Exp. Sta. Bul. 99.

(46) HUTCHINSON, H. B. 1913 Die Wirkung von gebranntem Kalk auf den Boden. *In* Chem. Ztg., Bd. 37, p. 251.

(47) HUTCHINSON, H. B., AND MACLENNAN, K. 1914 The relative effect of lime as oxide and carbonate on certain soils. *In* Jour. Agr. Sci., vol. 6, pt. 3, p. 302.

(48) HUTCHINSON, H. B., AND MACLENNAN, K. 1915 Studies on the lime-requirements of certain soils. *In* Jour. Agr. Sci., vol. 7, pt. 1, p. 75–106.

(49) JOHNSON, S. W. 1879 How Crops Grow. P. 315.

(50) JOHNSTON, J. F. W. 1849 The Use of Lime. 260 p.

(51) KELLY, W. P. 1912 The effects of $CaCO_3$ and $MgCO_3$ on some biological transformations of nitrogen in soils. *In* Univ. Cal. Pub. Agr. Sci., vol. 1, no. 3, p. 39–49.

(52) KELLY, W. P. 1915 Ammonification and nitrification in Hawaiian soils. Hawaii. Agr. Exp. Sta. Bul. 37.

(53) KOCH, A. 1907 The feeding of plants by the free-living nitrogen-fixing bacteria of the soil. *In* Mitt. Deut. Landw. Gesel., Bd. 22, No. 12, p. 117–121.

(54) KOCH, G. P. 1917 Potassium salts influencing biological activities. Doctor's Thesis, Rutgers College, New Brunswick, N. J.

(55) KOSSOVITCH, P., AND TRETJAKOV, I. 1902 On the influence of calcium carbonate on the progress of decomposition of organic matter. *In* Zhur. Opuitin. Agron. (Russ. Jour. Exp. Landw.), vol. 3, p. 450–484.

(56) KRAINSKI, A. 1908 Azotobacter chroococcum, etc. *In* Zhur. Opuitin. Agron. (Russ. Jour. Exp. Landw.), vol. 9, p. 689–749.

(57) KRUEGER, W. 1908 Beitrag zur Unters d. Sticksstoffumsetungen im Boden. Inaug. Diss. Konigsberg. *Abs. in* Centbl. Bakt. (etc.), Bd. 23 (1909), p. 236.

(58) LAUDER, A., FAGAN, T. W., AND STEELE, J. T. 1915 Experiment with lime and waste carbonate of lime, 1913–1914. *In* Edinb. and East of Scot. Col. Agr. Rpt. 1915, p. 10.

(59) LE BLANC, M., AND NOVOTONY, K. 1906 Cauticizing of alkali carbonates by lime. *In* Ztschr. Anorg. Chem., Bd. 51, p. 181.

(60) LEMMERMAN, O. A., FISCHER, H., et. al. 1909 Bacteriological-chemical investigations. *In* Landw. Jahrb., Bd. 38, p. 319–364.

(61) LINT, H. C., AND COLEMAN, D. A. 1916 Sources of error in soil bacteriological analysis. *In* Soil Sci., vol. 2, p. 157–162.

(62) LIPMAN, J. G. 1908 Report of soil chemist and bacteriologist. *In* N. J. Agr. Exp. Sta. Ann. Rpt. 1908, p. 170–186.

(63) LIPMAN, J. G. 1912 Miscellaneous vegetation experiments. N. J. Agr. Exp. Sta. Bull. 250.

(64) LIPMAN, J. G., AND BLAIR, A. W. 1913 Field experiments on the availability of nitrogenous fertilizers. N. J. Agr. Exp. Sta. Bul. 260, p. 33.

(65) LIPMAN, J. G., AND BROWN, P. E. 1907 Report of the soil chemist and bacteriologist. *In* N. J. Agr. Exp. Sta. Ann. Rpt. 1907, p. 186.

(66) LIPMAN, J. G., BROWN, P. E., AND OWEN, I. L. 1900 Relation of lime to ammonification. *In* N. J. Agr. Exp. Sta. Ann. Rpt. 1910, p. 89–124.

(67) LIPMAN, J. G., BROWN, P. E., AND OWEN, I. L. 1911 Experiments on ammonia and nitrate formation in soils. *In* Centbl. Bakt. (etc.), Abt. 2, Bd. 30, p. 156–181.

(68) LÖHNIS, F., AND GREEN, H. H. 1914 Ammonification and nitrification in soil and solution. In Centbl. Bakt. (etc.), Abt. 2, vol. 40, p. 457–479.

(69) LYON, T. L., AND BIZZELL, J. A. 1910 Availability of soil nitrogen in relation to the basicity of the soil and to the growth of legumes. *In* Jour. Indus. Engin. Chem., vol. 2, p. 313–315.

(70) LYON, T. L., AND BIZZEL, J. A. 1916 Calcium, magnesium, potassium and sodium in the drainage water from limed and unlimed soil. *In* Jour. Amer. Soc. Agron., vol. 8, no. 2, p. 81.

(71) MACINTIRE, W. H., WILLIS, L. G., AND HARDY, J. I. 1914 The non-existence of magnesium carbonate in humid soils. Tenn. Agr. Exp. Sta. Bul. 107.

(72) MCBETH, I. G., AND WRIGHT, R. C. 1912 Certain factors limiting nitrification. *In* Science n. s., vol. 35, no. 897, p. 392.

(73) MEYER, D. 1914 Versuche uber die Wirkung Verschiedner Kalkformen. *In* Illus. Landw. Ztg., Bd. 34, No. 61, p. 571–572.

(74) MILLER, F. 1914 Über den Einfluss des Kalkes auf die Bodenbakterien. *In* Ztschr. Garungsphysiol., Bd. 4, p. 194–206.

(75) MILLER, N. H. J. 1902 The amounts of nitrogen as ammonia and as nitric acid, and of chlorin in the rain water collected at Rothamsted. *In* Proc. Chem. Soc. London, vol. 18, p. 88–90.

(76) MOOERS, C. A. 1913 Liming for Tennessee soils. Tenn. Agr. Exp. Sta. Bul. 97.

(77) MORSE, F. W 1915 The effect on a crop of clover of liming the soil. Mass. Agr. Exp. Sta. Bul. 161.

(78) MORSE, F. W., AND CURRY, B. E. 1909 A study of the reactions between the manurial salts and clays, mucks and soils. N. H. Agr. Exp. Sta. Rpt. 1907–1908, p. 271–293.

(79) MÜLLER, P. E., AND WEIS, F. 1907 Über die Einwirkung des Kalkes auf Buchenrohhumus. *In* Naturw. Ztschr. Land u. Forstw., Bd. 5, p. 52–65.

(80) NEUBERTH 1900 The action of burnt lime and marl on light and sandy upland soils. *In* Deut. Landw. Presse, Bd. 27, No. 79, p. 983.

(81) NEUFFER, K. H. 1898 Das Kalksteinmehl im Dienste der Landwirtschaft.

(82) OWEN, W. L. 1908 The effect of carbonates on nitrification. Ga. Agr. Exp. Sta. Bul. 81 (Tech. Ser., no. 1).

(83) PATERSON, J. W., AND SCOTT, P. R. 1912 The relation of lime to soil fertility. *In* Jour. Dept. Agr. Victoria, vol. 10, p. 593.

(84) PATTERSON, H. J. 1894 Lime, sources and relation to agriculture. Md. Agr. Exp. Sta. Bul. 66, p. 127–130.

(85) PATTERSON, H. J. 1906 Investigations on the liming of soils. Md. Agr. Exp. Sta. Bul. 110, p. 16–21.

(86) PECK, S. S. 1910 Some biochemical investigations of Hawaiian soils, etc. Hawaiian Sugar Planters' Sta., Agr. and Chem. Bul. 34.

(87) PECK, S. S. 1911 Lysimeter experiments. Hawaiian Sugar Planters' Sta., Agr. and Chem. Bul. 37.

(88) PETER, A. M., AND JONES, S. C. 1912 The soils of Webster County. Ky. Agr. Exp. Sta. Bul. 162.

(89) PETERSON, P. 1871 Über den Einfluss des Mergels auf die Bildung von Kohlensäure und Salpetersäuren in Ackerboden. *In* Landw. Vers. Sta., Bd. 1–3, p. 155–175.

(90) PFEIFFER, T. 1908 Fixation of ammoniacal nitrogen by zeolites in soils. *In* Mitt. Landw. Inst. Breslau, Bd. 4, No. 3, p. 321.

(91) PORTER, E., AND GAUT, R. C. 1908 Report of experiments on the liming of meadow land. 1905–1908. County Council Lanc., Ed. Comm., Agr. Dept., F. B. 8.

(92) Rhode Island Agricultural Experiment Station. 1911 Extension News Service, vol. 1, no. 8.

(93) RIGGL 1912 Zur Kalkdüngung. *In* Illus. Landw. Ztg., Bd. 32, p. 855.

(94) ROTHERT 1915 Schädigung des Pflazenwachstums durch Atzkalk. *In* Jour. Landw., Bd. 63, Heft 111, p. 227–233.

(95) SAUL, M. 1904 Stückkalk oder Gemahlener Kalk in seiner Anwendung als Düngekalk? *In* Deut. Landw. Presse, Bd. 31, No. 36, p. 321–322.

(96) SCALES, F. M. 1915 Relation of lime to production of nitrates and mineral nitrogen. *In* Science, vol. 42, no. 1079, p. 317.

(97) SCHLOESING, T. 1872 Sur la dissolution du carbonate de chaux par l'acide carbonique. *In* Compt. Rend. Acad. Sci. [Paris], t. 74, p. 1552.

(98) SCHREINER, O., AND FAILYER, G. H. 1906 Colorimetric, turbidity and titration methods used in soil investigations. U. S. Dept. Agr. Bur. Soils Bul. 31.

(99) SIMMERMACHER, W. 1912 The action of calcium carbonate in the fertilizing of oats with mono- and dicalcium phosphate. *In* Landw. Vers. Sta., Bd. 77, p. 441.

(100) STEVENSON, W. H., AND WESTON, E. B. 1908 Clover growing on the loess and till soils of southern Iowa. Iowa Agr. Exp. Sta. Bul. 98.

(101) STREBUT, A. 1907 The effect of lime on nitrogen fertilizers. *In* Fühling's Landw. Ztg., Bd. 56, No. 19, p. 669–676.

(102) TACKE, B. 1897 Über die Bestimmung der freien Humussäuren im Moorboden. *In* Chem. Ztg., Bd. 21, p. 174–175.

(103) TAKEUCHI, T. 1907 Can calcium carbonate cause loss of ammonia by evaporation from the soil. *In* Bul. Col. Agr. Tokyo Imp. Univ., vol. 7, no. 3, p. 433.

(104) THOMAS, W., AND FREAR, W. 1915 The importance of fineness of subdivision to the utility of crushed limestone as a soil amendment. *In* Jour. Indus. Engin. Chem., vol. 7, no. 12, p. 1041.

(105) THORNE, C. E. 1905 Liming the soil. Ohio Agr. Exp. Sta. Bul. 159.

(106) THORNE, C. E. 1914 Liming the land. Ohio Agr. Exp. Sta. Bul. 279.

(107) TREADWELL, F. P., AND REUTER, M. 1898 Über die Löslichkeit der Bikarbonate des Calciums und Magnesiums. *In* Ztschr. Anorg. Chem., Bd. 17, p. 170.

(108) TRUOG, E. 1916 A new apparatus for the determination of soil carbonates and new methods for the determination of soil acidity. *In* Jour. Indus. Engin. Chem., vol. 8, no. 4, p. 341.

(109) VEITCH, F. P. 1905 Summary of experiments on the relation of soil acidity to fertility. U. S. Dept. Agr. Bur. Chem. Bul. 90, p. 183–187.

(110) VOELCKER, J. A. 1910 The influence of lime and magnesia in different forms on wheat. *In* Jour. Roy. Agr. Soc. England, vol. 71, p. 346–348.

(111) VOELCKER, J. A. 1914 Grass and pot culture experiments. *In* Jour. Roy. Arg. Soc. England, vol. 75, p. 306–322.

(112) VOGEL, J. 1911 Untersuchungen uber den Einfluss von kohlensauren Kalk auf die Umwandlung von Ammoniakstickstoff und Nitratstickstoff. *In* Mitt. Kaiser Wilhelms Inst. Landw. Bromberg, Bd. 3, p. 330–350.

(113) VOORHEES, E. B., LIPMAN, J. G., AND BROWN, P. E. 1907 Chemical and bacteriological effects of liming. N. J. Agr. Exp. Sta. Bul. 210.

(114) WARINGTON, R. 1866 Researches on the phosphates of calcium, etc. *In* Jour. Chem. Soc. [London], vol. 19, p. 296.

(115) WHEELER, H. J. 1910 Needs of Rhode Island soils. R. I. Agr. Exp. Sta. Bul. 139.

(116) WHIPPLE, G. C., AND MAYER, A., JR. 1906 The solubility of calcium carbonate and of magnesium hydroxide and the precipitation of these salts with lime water. *In* Jour. Infect. Diseases, Suppl. 2, p. 151–165.

(117) WHITE, J. W. 1912 Studies in acid soils. Penn. Agr. Exp. Sta. Rpt. 1912–1913. Separate no. 5.

(118) WHITE, J. W. 1913 The effects of pure and magnesium limestone upon the formation of nitrates on a very acid soil. Penn. Agr. Exp. Sta. Ann. Rpt. 1913–1914, p. 79.

(119) WHITSON, A. R., AND WEIR, W. W. 1913 Soil acidity and liming. Wis. Agr. Exp. Sta. Bul. 230.

(120) WILKINS, L. K. 1914 The relation of fineness of division of limestone to the rate of decomposition of organic matter in soil as measured by the evolution of $CO_2$ and the formation of nitrates. Unpublished B. S. thesis, Rutgers College Library.

(121) WOLLNY 1897 Die Zersetsung der organischen Stoffe, etc., p. 128–134.

(122) WRIGHT, R. C. 1916 Growing plants in large containers under control conditions. *In* Jour. Amer. Soc. Agron., vol. 8, no. 2, p. 113.

(123) WRIGHT, R. P. 1911 Best method of applying lime to arable land. *In* West of Scot. Agr. Col. Bul. 55, p. 197–216.

(124) YOKOYAMA, H. 1909 Is artificial calcium carbonate more effective than limestone meal? *In* Jour. Col. Agr. Tokyo Imp. Univ., vol. 1, p. 180.

## PLATE I

Galvanized iron pots permitting collection of drainage water, cropped to barley.

*Treatment: 11, 12,* Minerals + 12 grams $(NH_4)_2SO_4$; *13, 14,* Minerals + 12 grams $(NH_4)_2SO_4$ + 20–40 mesh limestone; *15, 16,* minerals + 12 grams $(NH_4)_2SO_4$ + 60–80 mesh limestone.

12
13
14
15
16

# THE YIELD AND NITROGEN CONTENT OF SOYBEANS AS INFLUENCED BY LIME[1]

J. G. LIPMAN AND A. W. BLAIR
*From the New Jersey Agricultural Experiment Station*

Received for publication January 15, 1917

In earlier papers (2, 4) the authors have called attention to some of the factors which may influence the protein content of soybeans. It was pointed out, for example, that the shelled beans from limed plots contained a higher percentage of nitrogen than those grown on similar unlimed plots, and that this was true for all the varieties experimented with.

A continuation of the work has confirmed the earlier results, and has further shown that what is true of the shelled beans is also generally true of the top part of the plant when harvested as forage or as dry stalks, and of the roots with their accompanying nodules.

In this connection it was suggested by the senior author that a count of the nodules on the roots of plants from limed and unlimed plots might throw some light on the subject.

## METHOD OF CONDUCTING THE EXPERIMENT

The soil on which this experiment was carried out is a loam of fair quality, but previous to 1908 it had been neglected. In the spring of 1908 certain of the plots received a treatment of ground limestone at the rate of 2000 pounds per acre, and in 1913 a further treatment at the rate of 4000 pounds per acre. Other plots referred to as the "unlimed plots" have received no lime treatment. All plots receive annual applications of acid phosphate at the rate of 400 to 600 pounds per acre and muriate of potash at the rate of 100 to 200 pounds per acre. Since 1908 the plots have been used for leguminous crops almost entirely and as this crop has been soybeans for the past four years the soil is now well inoculated, as shown both by luxuriant growth and the abundance of nodules on the roots. The soil originally contained about 0.09 to 0.10 per cent of total nitrogen. The beans were planted about May 8 in rows 33 inches apart. Germination was good and during the season they received the usual attention in the way of cultivation and hoeing.

## RESULTS FROM CROP HARVESTED AS FORAGE

When the pods were well set, but before ripening had commenced, twelve plants were carefully removed from one of the rows of each of 6 varieties that

[1] The authors are indebted to Mr. H. C. McLean for the chemical analyses and to Mr. L. K. Wilkins for supervision of the field work.

were growing on limed and unlimed plots, and a count was made of the number of nodules per plant. This count showed an average of 83.6 nodules per plant for the six varieties grown on limed plots, and 50 nodules per plant for the same varieties on unlimed plots.

The number of nodules varied rather widely with the different varieties, but with all varieties the number was greater on the limed than on the unlimed plots. This would seem to substantiate further the generally accepted view that well limed soils, other things being equal, present more favorable conditions for inoculation, and therefore for the accumulation of atmospheric nitrogen by means of leguminous crops, than soils that are distinctly acid. The results obtained from the six varieties harvested as above described are given in table 1.

With one exception only, the yield of dry matter, both tops and roots, is greater for the limed than for the unlimed plots. Likewise, with only one exception, the percentage of nitrogen in the dry matter, both tops and roots, is greater for the limed than for the unlimed plots.

The average nitrogen content of the tops for the six varieties is—limed, 3.08 per cent, and unlimed 2.67 per cent; and the average for the roots is—limed, 1.47 per cent, and unlimed, 1.24 per cent. It is thus shown that decidedly more nitrogen has been accumulated on the limed than on the unlimed plots. This is shown in the two columns at the right—the average amount in a single plant, root and top, for the limed plots being 0.71 gram and for the unlimed plots 0.43 gram.

Manchu for the limed plots shows the highest percentage of nitrogen, both roots and tops, and also the largest amount of nitrogen recovered in the crop.

## RESULTS FROM THE MATURED CROP

The beans on a portion of each one of the plots referred to above were allowed to ripen and were harvested by pulling up the entire plant. These were stood in bunches in the field until dry, when they were taken in and threshed and samples of both beans and stalks prepared for analysis. At the time of harvesting, the plants had shed practically all of the leaves so that these were left on the field in place of the roots. With the stalks were included the roots and also the bean hulls.

The dry weights, calculated to the acre basis, together with the results of the nitrogen determinations are shown in table 2.

Here, as with the forage, the yield of dry matter and percentage of nitrogen for both shelled beans and stalks, are, with slight exception, higher on the limed than on the unlimed plots. This, it may be pointed out, is in accordance with results secured in similar experiments conducted in 1914 (4) and 1915 (2).

The average yield of shelled beans for the six varieties (Cloud, Hollybrook, Ito San, Manchu, Ohio 9035 and Swan) on the limed plots is 19.3 bushels

TABLE 1

*The influence of lime on the number of nodules and percentage of nitrogen for soybeans when harvested as hay*

| VARIETY | PER PLANT—TOPS (AVERAGE OF TWELVE PLANTS) | | | | | | PER PLANT—ROOTS WITH NODULES (AVERAGE OF TWELVE PLANTS) | | | | | | | | NITROGEN RECOVERED IN ONE PLANT, ROOTS AND TOPS | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Limed | | | Unlimed | | | Limed | | | | Unlimed | | | | | |
| | Dry matter | Nitrogen | Nitrogen | Dry matter | Nitrogen | Nitrogen | Nodules per plant | Dry matter | Nitrogen | Nitrogen | Nodules per plant | Dry matter | Nitrogen | Nitrogen | Limed | Unlimed |
| | *grams* | *per cent* | *gram* | *grams* | *per cent* | *gram* | | *grams* | *per cent* | *gram* | | *grams* | *per cent* | *gram* | *gram* | *gram* |
| Cloud | 22.68 | 2.964 | 0.672 | 11.34 | 2.508 | 0.284 | 29.8 | 2.542 | 1.070 | 0.0272 | 9.4 | 1.650 | 1.107 | 0.0183 | 0.992 | 0.3023 |
| Hollybrook | 18.90 | 2.991 | 0.565 | 18.90 | 2.535 | 0.479 | 61.2 | 2.817 | 1.479 | 0.0417 | 28.3 | 2.733 | 1.274 | 0.0348 | 0.6067 | 0.5138 |
| Manchu | 26.46 | 3.402 | 0.900 | 17.01 | 2.745 | 0.467 | 82.8 | 2.600 | 1.851 | 0.0481 | 27.9 | 2.250 | 1.200 | 0.0270 | 0.9481 | 0.4940 |
| Medium yellow | 17.01 | 3.137 | 0.534 | 15.12 | 2.544 | 0.385 | 76.8 | 2.667 | 1.284 | 0.0342 | 52.4 | 2.158 | 1.088 | 0.0235 | 0.5682 | 0.4085 |
| Ohio 9035 | 22.68 | 2.745 | 0.623 | 17.01 | 2.572 | 0.437 | 161.9 | 3.417 | 1.637 | 0.0559 | 131.8 | 3.333 | 1.312 | 0.0437 | 0.6789 | 0.4807 |
| Swan | 22.68 | 3.210 | 0.728 | 11.34 | 3.092 | 0.351 | 89.0 | 2.667 | 1.479 | 0.0394 | 50.2 | 1.875 | 1.302 | 0.0244 | 0.7674 | 0.3754 |
| Average | 21.74 | 3.075 | 0.670 | 15.12 | 2.666 | 0.401 | 83.6 | 2.785 | 1.467 | 0.0411 | 50.0 | 2.333 | 1.214 | 0.0286 | 0.7114 | 0.4291 |

TABLE 2

*The influence of lime on the yield and nitrogen content of soybeans when harvested at maturity*

*Calculated on the acre basis*

| VARIETY | SOYBEAN SEEDS | | | | | | SOYBEAN STALKS | | | | | | TOTAL NITROGEN RECOVERED—SEEDS AND STALKS | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Limed | | | Unlimed | | | Limed | | | Unlimed | | | | |
| | Dry matter | Nitrogen | Nitrogen | Dry matter | Nitrogen | Nitrogen | Dry matter | Nitrogen | Nitrogen | Dry matter | Nitrogen | Nitrogen | Limed | Unlimed |
| | *bushels* | *per cent* | *pounds* | *bushels* | *per cent* | *pounds* | *pounds* | *per cent* | *pounds* | *pounds* | *per cent* | *pounds* | *pounds* | *pounds* |
| Baird | 20.80 | 7.025 | 87.66 | | | | 3,552 | 1.107 | 39.30 | | | | 126.96 | |
| Cloud | 19.00 | 5.744 | 65.94 | 13.13 | 5.540 | 43.68 | 2,052 | 0.608 | 12.48 | 2,012 | 0.499 | 10.04 | 78.42 | 53.72 |
| Ebony | 19.90 | 6.598 | 78.78 | | | | 2,406 | 1.626 | 39.12 | | | | 117.90 | |
| Edna | 21.85 | 6.394 | 83.82 | | | | 2,589 | 0.907 | 23.46 | | | | 107.28 | |
| Guelph | 18.40 | 5.698 | 62.88 | | | | 2,492 | 0.618 | 15.42 | | | | 78.30 | |
| Hollybrook | 14.27 | 6.412 | 54.88 | 15.13 | 6.023 | 54.72 | 2,344 | 0.718 | 16.80 | 1,492 | 0.479 | 7.15 | 71.68 | 61.87 |
| Ito San | 17.50 | 6.032 | 63.32 | | | | 2,670 | 0.788 | 21.04 | | | | 84.36 | |
| Manchu | 19.80 | 6.561 | 77.92 | 12.27 | 5.642 | 41.52 | 1,612 | 0.519 | 8.40 | 864 | 0.608 | 5.25 | 86.32 | 46.77 |
| Manhattan | 16.25 | 6.236 | 60.78 | | | | 2,325 | 0.758 | 17.64 | | | | 78.42 | |
| Medium yellow | 18.00 | 5.837 | 63.04 | 12.87 | 5.522 | 42.64 | 2,120 | 0.628 | 13.28 | 1,228 | 0.618 | 7.59 | 76.32 | 50.23 |
| Ohio 9035 | 23.06 | 5.884 | 81.44 | 14.40 | 5.392 | 46.56 | 2,216 | 1.446 | 32.08 | 1,536 | 0.947 | 14.55 | 113.52 | 61.11 |
| Swan | 21.67 | 6.461 | 84.00 | 11.33 | 6.245 | 42.48 | 1,900 | 0.828 | 15.76 | 920 | 0.539 | 4.96 | 99.76 | 47.44 |
| Tarheel | 9.83 | 5.642 | 33.28 | | | | 4,610 | 0.967 | 44.56 | | | | 77.84 | |
| Wilson | 25.55 | 5.726 | 87.78 | | | | 2,667 | 0.618 | 16.50 | | | | 104.28 | |
| Average | 19.30* | 6.150* | 71.20* | 13.19 | 5.727 | 45.27 | 2,041* | 0.791* | 16.47* | 1,342 | 0.615 | 8.26 | 87.67* | 53.52 |

* Average of the six varieties having corresponding unlimed plots. The other varieties did not have corresponding unlimed plots.

per acre and for the same varieties on the unlimed plots it is 13.2 bushels per acre. The increase on the limed plots of 6.1 bushels per acre would more than pay for the lime, but since this is the fourth crop of beans since the last application of lime and all the crops were benefitted by the liming, the lime has been paid for long ago, and the 6.1 bushels represent net profit in addition to the increase in amount of stalks, which have some value. In 1915 seven varieties on limed plots yielded, on an average, 6.5 bushels more than the same varieties on unlimed plots.

Out of the fourteen varieties in 1916, only four fell below a yield of 18 bushels per acre on the limed plots, while the Wilson gave a yield of 25.5 bushels and Ohio 9035, Swan, Edna and Baird yielded between 20 and 25 bushels per acre. The low yield secured with Tarheel must no doubt be attributed to the fact that this is a slow-growing late-maturing variety; had heavy frosts come early the yield would have been less, and possibly no beans would have matured. This variety, however, if given a long growing season, makes a heavy stem and leaf growth and for this reason would make an excellent green-manure crop where land is not to be otherwise used during the summer and fall. The heavy growth is indicated by a yield of over two tons per acre of dry stalks. The yield of dry stalks is, without exception, larger on the limed than on the unlimed plots. For the six varieties that have corresponding unlimed plots, the yield is approximately 50 per cent greater, while the total nitrogen for these same varieties is just about 100 per cent greater.

With only one exception (stalks from Manchu), the percentage of nitrogen in beans and stalks from limed plots is greater than it is from corresponding unlimed plots. This is in accord with the results of the past three years, reference to which has already been made. This is likewise in accord with results secured at the Massachusetts Agricultural Experiment Station with red clover (5).

It is thus shown that the use of lime has consistently increased the yield of beans and stalks and has also given a feeding material richer in protein than that grown on unlimed plots. Special interest attaches to the total nitrogen recovered in seeds and stalks for the limed and unlimed plots. The lowest yield for a limed plot—71.68 pounds per acre—is greater by about 10 pounds than the highest yield on an unlimed plot.

The following varieties, limed, gave a yield of nitrogen above 100 pounds: Baird, Ebony, Edna, Ohio 9035 and Wilson, with Swan almost at the 100-pound mark. The average for the six unlimed plots is 53.52 pounds, while the average for the six corresponding limed plots is 87.67, or an increase of more than 50 per cent.

It is not possible to say how much of this nitrogen was gained from the air, and how much from the soil. It has been shown, however, from a number of experiments covering a period of eight or nine years (3) that non-leguminous crops grown on nearby plots, where the soil is practically the same as that of the soybean plots, when unaided by nitrogenous fertilizers or

green manures, are not able to gain from this soil more than about 20 to 25 pounds of nitrogen per acre, and while it does not necessarily follow as true, it seems reasonable to assume that the soybeans would probably not take from the *soil* more nitrogen than the other crops. This would mean, on an average, about 65 pounds of nitrogen per acre taken from the air, and if allowance is made for the leaves which were left on the field, it would probably mean more. This is in fair agreement with results secured by means of cylinder experiments in an indirect way, where leguminous crops were used as a source of nitrogen in comparison with nitrate of soda and stable manure (1).

This points to the soybean as a plant especially efficient as an accumulator of atmospheric nitrogen and emphasizes the desirability of its wider use as a means of maintaining the nitrogen and humus supply of the soil.

## SUMMARY

1. Earlier experiments have shown that when soybeans are grown on limed and unlimed plots, the liming not only increases the yield of dry shelled beans, but also the nitrogen content of the beans.

2. The results secured in 1916 confirm the earlier experiments and also show that liming increases the yield of forage when the crop is harvested either as hay or as dry stalks, and also increases the percentage of nitrogen in these materials.

3. A count of the nodules on roots of plants taken up about the time the pods were fairly well filled showed an average of 83.6 nodules per plant on the limed plots and 50 nodules per plant on the unlimed plots.

4. The average yield of shelled beans on the unlimed plots was 13.2 bushels per acre and the average on the corresponding limed plots was 19.3 bushels. The average percentage of nitrogen in the beans for unlimed plots was 5.73 per cent and for the limed plots 6.15 per cent. In 1915 the corresponding percentages were 5.92 and 6.41, and in 1914 they were 6.21 and 6.70.

5. The average yield of stalks was, for unlimed plots, 1342 pounds per acre, and for corresponding limed plots, 2041 pounds per acre. The average nitrogen content of stalks from the unlimed plots was 0.615 per cent and from the corresponding limed plots, 0.791 per cent.

6. The average total yield of nitrogen recovered in the crop from the unlimed plots was 53.52 pounds per acre and the average for the corresponding limed plots 87.67 pounds per acre.

7. Reasoning from the amount of nitrogen which non-leguminous crops, without the aid of commercial fertilizers or green manures, have drawn from nearby plots where the soil is similar, it is believed that as much as 65 pounds of this nitrogen was taken from the air.

## LITERATURE CITED

(1) LIPMAN, J. G., AND BLAIR, A. W. 1916 Cylinder experiments relative to the utilization and accumulation of nitrogen. N. J. Agr. Exp. Sta. Bul. 289.

(2) LIPMAN, J. G., AND BLAIR, A. W. 1916 Factors influencing the protein content of soy-beans. *In* Soil Sci., vol. 1, no. 2, p. 171–178.

(3) LIPMAN, J. G., AND BLAIR, A. W. 1917 Influence of lime upon the yield of dry matter and nitrogen content of alfalfa. *In* N. J. Agr. Exp. Sta. 37th Ann. Rpt. (1916) (soon to appear).

(4) LIPMAN, J. G., BLAIR, A. W., MCLEAN, H. C., AND WILKINS, L. K. 1914 Factors influencing the protein content of soy-beans. N. J. Agr. Exp. Sta. Bul. 282.

(5) MORSE, F. W. 1915 The effect on a crop of clover of liming the soil. Mass. Agr. Exp. Sta. Bul. 161.

# FERMENTATION OF MANURE TREATED WITH SULFUR AND SULFATES: CHANGES IN NITROGEN AND PHOSPHORUS CONTENT

J. W. AMES AND T. E. RICHMOND

*Ohio Agricultural Experiment Station*

Received for publication March 16, 1917

## INTRODUCTION

The loss of nitrogen from decomposing manure under certain conditions of fermentation proceeds rapidly. Wagner (21) has shown that the nitrogen of urine can be transformed into ammonium compounds within 48 hours after being voided. Pfeiffer (14), Wildt (17) and Deherain (7) have reported results showing that under certain conditions of fermentation manures may lose as much as 42 per cent of their total nitrogen as the free element. Niklewski (13) states that doubt is unfounded as to the liberation of elementary nitrogen from decomposing manure in consequence of the cooperation between nitrifying and denitrifying bacteria. Dietzell (8), Bjorn-Anderson (2) and Pfeiffer (14) have proved that the addition of acid phosphate to manure will prevent the liberation of nitrogen. Gerlach (9) has recently reported that the action of gaseous ammonia upon acid phosphate results in the formation of ammonium sulfate and calcium phosphate. This is evidence that the conservation of nitrogen in manure treated with acid phosphate is the result of a combination between ammonia formed and calcium sulfate furnished by the acid phosphate, rather than the prevention of the formation of ammonia.

The treatment of manure with gypsum is also a common practice. Experiments in which this material was added to both solid and liquid manure have been reported by Schneidewind (15), Severin (16), Dietzell (8) and Bohme (3). The results show that a loss of more than 50 per cent of the total nitrogen may occur from untreated manure, but that when 5 to 10 per cent of calcium sulfate is added a very small loss of nitrogen occurs and that the nitrogen, in the case of urine, is almost completely changed to ammonium sulfate, and held as such. Work reported by the Copenhagen Station (6) and by Stoklasa (18) shows that the addition of sulfuric acid reduced the loss of nitrogen from 75 per cent in untreated manure to almost nothing. Hall (10) and Schneidewind have each called attention to the harmful effects that may be caused by the application of gypsum-treated manure to the soil. The claim is made that sulfides produced by reduction of sulfate are poisonous

to growing plants. The results from the barnyard manure test plots of the Ohio Agricultural Experiment Station, where gypsum mixed with manure at the rate of 40 pounds per ton has been used since 1897, show no such effect, but, on the other hand, a gain over the plots treated with manure alone. It is also shown by these results (reported in Circular 144, Ohio Agr. Exp. Sta.) that the manure designated as "stall manure" has produced larger crop yields than "yard manure," thus proving that prevention of loss by leaching is necessary to realize the fullest return from reinforcing materials.

The use of elementary sulfur as a reinforcing material for manure has not as yet received much attention. Street (19) in 1900 reported experiments planned for the purpose of studying changes which nitrogen of barnyard manure undergoes in presence of various fertilizing materials. Results of this work continued during two years showed that sulfur either in the form of commercial flowers of sulfur or as sulfate of iron has a marked effect in decreasing the loss of ammonia.

Cook and Hutchinson (5) report that the addition of 1 pound of sulfur per bushel of manure greatly reduced the number of flies hatched from manure, but that such heavy applications applied to the soil would undoubtedly have an injurious effect on plant growth.

J. C. Lipman and associates (11) have recently called attention to the benefit to be gained from treatment of compost heaps with sulfur and "floats" by furnishing available phosphorus.

## EXPERIMENTAL

For the purpose of studying the influence of sulfur and other materials upon the fertilizing value of manure as measured by changes in its composition and the response made by growing crops, experiments with reinforcing materials were started at the Ohio Agricultural Experiment Station in May, 1915. The first experiment reported deals with the effects of sulfur, calcium sulfate and acid phosphate upon the changes occurring in solid horse manure. Data pertaining to nitrogen, phosphorus and sulfur are included. Manure which is being treated in larger containers and under the conditions described is being applied after fermentation to small plots to determine the influence of the several treatments on soil conditions and plant growth.

Under the second experiment the effect of the same reinforcing materials on the nitrogen content of cow's urine is shown.

### *Experiment with solid manure*

The horse manure used in this experiment had accumulated for about one week in box stalls, fine cut wheat straw being used as litter to facilitate mixing and sampling. The manure was mixed and all lumps pulverized to insure a complete intermixture of added materials. Four lots of about 30 pounds each were weighed out and treated as follows: to no. 1 was added

342 grams of acid phosphate; to no. 2, 407 grams of calcium sulfate; and to no. 3, 90 grams of flowers of sulfur; the fourth lot was left untreated. After addition of the different materials the samples were again thoroughly mixed and appeared to be of uniform structure. About 25 pounds of each of these mixtures was placed in weighed containers and the remainder saved for analysis.

The containers were 15 inches in diameter and 19 inches high, and were made with a cover that allowed a paraffin seal to be made. The only air admitted was through a glass tube extending to the bottom of the container through a rubber stopper in the center of the cover. The weight of container and contents was then obtained. Air was drawn through the apparatus for

TABLE 1

*Changes in weight and loss of dry matter*

| | MANURE AND ACID PHOSPHATE | MANURE AND SULFUR | MANURE AND CALCIUM SULFATE | MANURE UNTREATED |
|---|---|---|---|---|
| | *grams* | *grams* | *grams* | *grams* |
| Total weight of mixture at beginning | 8,471 | 11,632 | 11,726 | 11,211 |
| Total weight of mixture at end | 8,201 | 11,392 | 11,176 | 10,505 |
| Total weight of dry matter at beginning | 3,617 | 4,210 | 4,362 | 4,047 |
| Total weight of dry matter at end | 2,829 | 3,441 | 3,408 | 2,731 |
| Percentage of dry matter lost | 21.78 | 18.2 | 21.87 | 32.5 |
| Total weight of moisture at beginning | 4,854 | 7,422 | 7,364 | 7,164 |
| Total weight of moisture at end | 5,372 | 7,951 | 7,768 | 7,774 |
| Gain in moisture | 518 | 529 | 404 | 610 |
| Percentage of moisture at beginning | 57.3 | 63.9 | 52.8 | 63.9 |
| Percentage of moisture at end | 65.5 | 69.8 | 69.5 | 74.0 |

3 hours on 3 days a week by means of a Crowell rotary pump, which drew 100 liters per hour through each container. The air drawn through the containers was first passed through three wash bottles containing sulfuric acid and sodium hydroxide and water, in the order named, and then conducted through the glass tubes to the bottom of the containers. The air was drawn from the outlet in the top of the container and passed first into a trap and then through sulfuric acid and sodium hydroxide solutions for absorption of ammonia and hydrogen sulfide.

*Loss of organic matter.* After standing at room temperature from May 7, 1915 to January 14, 1916, the cans and contents were weighed and moisture determinations made immediately. The data for moisture content and loss of organic matter are given in table 1.

The results show that there was an appreciable loss of dry matter due to the destructive processes occurring during the fermentation of manure under the conditions to which it was subjected in this experiment.

The manure treated with flowers of sulfur lost only 18.2 per cent of its dry matter as compared with a loss of 32.5 per cent from the untreated manure. The samples treated with acid phosphate and calcium sulfate lost approximately the same amounts and only slightly more than the sulfur-treated manure. This is a reduction of more than 44 per cent in the loss of dry matter from the manure when treated with sulfur, and a reduction of more than 32 per cent by the addition of either acid phosphate or calcium sulfate.

These treatments appear to have been unfavorable for the development of cellulose-dissolving bacteria, which rapidly destroy cellulose (12), the products formed by their activities being immediately used by other microorganisms with the liberation of carbon dioxide and methane.

TABLE 2

*Nitrogen loss and changes*

| | MANURE AND ACID PHOSPHATE | MANURE AND SULFUR | MANURE AND CALCIUM SULFATE | MANURE UNTREATED |
|---|---|---|---|---|
| | *grams* | *grams* | *grams* | *grams* |
| Weight of total nitrogen at beginning | 66.0738 | 94.8008 | 89.1176 | 96.4146 |
| Weight of total nitrogen at end | 63.9678 | 91.1360 | 86.0552 | 86.1410 |
| Weight of nitrogen lost | 2.1060 | 3.6648 | 3.0624 | 10.2321 |
| Weight of nitrogen recovered as ammonia | 0.0026 | 0.0103 | 0.0296 | 0.0415 |
| Weight of nitrogen lost as free element or experimental error | 2.1034 | 3.6545 | 3.0328 | 10.2321 |
| Percentage of total lost | 3.1 | 3.8 | 3.4 | 10.6 |
| Weight of water-soluble nitrogen at beginning | 30.83 | 42.80 | 43.85 | 41.14 |
| Weight of water-soluble nitrogen at end | 6.31 | 14.69 | 14.52 | 12.81 |
| Percentage of original water-soluble nitrogen changed | 79.53 | 65.6 | 66.88 | 68.8 |

A dried and ground sample of the sulfur-treated manure at the end of the experiment gave an acid water extract, while that of the untreated manure gave an alkaline reaction. The fact that the manures contained more water at the end of the experiment than at the beginning indicates that the bacteria had optimum working conditions so far as the moisture content was concerned.

*Changes in nitrogen content.* The amounts of different forms of nitrogen found in the manure before and after fermentation are given in table 2. Since a small amount only of the total nitrogen content was evolved as ammonia from the untreated and treated manure, it appears that the conditions of fermentation existing during the experiment were not favorable for the liberation of appreciable quantities of ammoniacal nitrogen. The weights of nitrogen evolved from the different samples as ammonia and as free nitrogen are shown in table 2.

*Total nitrogen.* The three lots of treated manure have lost approximately the same amounts of total nitrogen, viz., 3.5 per cent. This figure compared with a loss of 10.5 per cent in the untreated manure shows that there was a conservation of nitrogen by the reinforcing materials used. Since only a small amount of ammonia was recovered, nitrogen must have been lost as free nitrogen.

The treatments either have made conditions unfavorable for ammoniacal fermentation or have been efficient in preventing the escape of ammonia which may have been produced. The latter view appears to furnish the more correct explanation when the results obtained are considered in more detail.

The acid phosphate treatment, no doubt, furnished sufficient calcium sulfate to combine with ammonia as it was produced, forming ammonium sulfate. A reaction may also have taken place to some extent between monocalcium phosphate and ammonia. In the sample treated with calcium sulfate the ammonia formed would also be held as ammonium sulfate. Evidence of the effectiveness of calcium sulfate in this respect is furnished by results obtained in the second experiment reported where liquid manure was treated with calcium sulfate. The fact that the manure treated with sulfur developed acidity equivalent to 24 grams of sulfuric acid during fermentation is evidence that this acidity may have been responsible for the retention of nitrogen as ammonia where sulfur was used in this experiment. The conditions maintained were evidently such that the loss of free nitrogen was brought about by denitrification processes developing between periods of aeration or during the latter part of the experiment, during which time the containers were aerated to a less extent than previously.

*Water-soluble and non-proteid nitrogen.* There is, under all conditions of manure fermentation, a transformation of water-soluble nitrogen to insoluble nitrogen, which is due to the utilization of ammonia and other soluble compounds of nitrogen by bacteria. In this experiment the effect of bacterial activities during fermentation of the untreated manure and that treated with gypsum and sulfur has been to change approximately 67 per cent of the water-soluble nitrogen originally present to some form insoluble in water. In the acid phosphate-treated manure there was a greater decrease in the water-soluble nitrogen content, about 80 per cent being changed to insoluble nitrogen. The supply of soluble phosphorus furnished may have favored the development of bacteria.

During fermentation about 70 per cent of the non-proteid nitrogen present in the untreated manure and that treated with sulfur and calcium sulfate at the beginning of the experiment has been changed to proteid nitrogen.

The decreased content of water-soluble nitrogen and the increase in proteid nitrogen shows the effect of fermentation on solubility and availability of the nitrogen compounds of manure.

The work of R. A. Berry (1) regarding these changes in nitrogen content

of manure during storage shows that the solubility of nitrogen is decreased considerably during fermentation.

*Changes in phosphorus.* The analytical data pertaining to changes in the solubility of phosphorus in the different lots of manure are given in table 3. With the exception of the manure treated with acid phosphate, the total phosphorus content is approximately the same.

*Water-soluble and citrate-insoluble phosphorus.* During the fermentation of untreated manure the solubility of the phosphorus was decreased 24 per

TABLE 3

*Changes in phosphorus*

| | MANURE AND ACID PHOSPHATE | MANURE AND SULFUR | MANURE AND CALCIUM SULFATE | MANURE UNTREATED |
|---|---|---|---|---|
| | *grams* | *grams* | *grams* | *grams* |
| Weight of phosphorus before and after fermentation | 27.72 | 14.55 | 14.28 | 14.12 |
| Weight of water-soluble phosphorus at beginning | 10.81 | 6.53 | 5.12 | 5.68 |
| Weight of water-soluble phosphorus at end | 4.05 | 4.23 | 2.21 | 4.28 |
| Percentage of original changed | 62.53 | 35.2 | 56.83 | 24.6 |
| Weight of phosphorus soluble in 0.2 per cent alkali at beginning | 6.59 | 9.05 | 10.00 | 8.57 |
| Weight of phosphorus soluble in 0.2 per cent alkali at end | 2.46 | 5.32 | 3.09 | 5.09 |
| Percentage of original changed | 62.67 | 41.2 | 69.10 | 40.6 |
| Weight of phosphorus soluble in 0.2 per cent acid at beginning | 21.92 | 4.57 | 4.24 | 3.74 |
| Weight of phosphorus soluble in 0.2 per cent acid at end | 29.79 | 7.69 | 7.77 | 8.69 |
| Weight of inorganic phosphorus at beginning | 8.18 | 2.12 | 2.35 | 1.94 |
| Weight of inorganic phosphorus at end | 12.23 | 6.47 | 2.40 | 5.03 |
| Weight of citrate-insoluble phosphorus at beginning | 4.38 | 2.88 | 2.84 | 3.54 |
| Weight of citrate-insoluble phosphorus at end | 3.77 | 2.78 | 2.30 | 2.13 |
| Percentage of original changed | 13.92 | 3.4 | 19.01 | 40.0 |

cent. The reduction of water-soluble phosphorus in the manure treated with acid phosphate was 62 per cent of the soluble phosphorus present at the beginning of the experiment. As the acid phosphate added contained 5.8 per cent of water-soluble phosphorus, the greater decrease in solubility was that of soluble phosphorus added. Calcium sulfate has actively decreased the solubility of phosphorus originally present. If depression of solubility of phosphorus in fermenting manure is due to the function which phosphorus performs in the life cycle of bacteria, it would seem that calcium sulfate had stimulated bacterial activities.

The amount of citrate-insoluble phosphorus in all the samples was less after fermentation than before, showing that during fermentation there has been a change from insoluble to citrate-soluble forms. The greatest change is found in the untreated sample, in which 40 per cent of the original citrate-insoluble phosphorus has been changed to forms soluble in neutral ammonium citrate solution. The acid phosphate and calcium sulfate samples show a change of 13 and 19 per cent, respectively; the change in manure treated with sulfur was very small. The results for water-soluble and citrate-insoluble phosphorus show that, while there has been a loss of water-soluble phosphorus during fermentation, at the same time there has occurred an increase of phosphorus soluble in neutral ammonium citrate. Similar results are reported by Tottingham and Hoffman (20).

*Inorganic phosphorus.* The results obtained for inorganic phosphorus by the method (4) used show that the amount present at the beginning of the experiment has been increased during fermentation, except in the case of the calcium sulfate-treated manure, where the same amount was found at the end of the experiment as at the beginning.

Solubility of phosphorus in 0.2 per cent sodium hydroxide solution has been reduced in all the samples by approximately 50 per cent of the amount originally present. Aside from having a solvent action on iron and aluminum phosphates possibly present, it would be expected that the action of dilute alkali would be greater on organic phosphorus compounds than on inorganic phosphates. There has been a decidedly increased solubility of phosphorus in 0.2 per cent hydrochloric acid, and this increase has been greater in the manure to which no phosphorus was added.

The comparative solubility of phosphorus in 0.2 per cent hydrochloric acid and water, before and after fermentation, is of interest as indicating a change from organic to inorganic phosphorus. With the exception of manure treated with acid phosphate, less phosphorus was soluble in 0.2 per cent acid than in water at the beginning of the experiment, while after fermentation the acid-soluble phosphorus exceeded the water-soluble. The reduced solubility of phosphorus in dilute alkali and the increased solubility in acid, considered in connection with results obtained for inorganic phosphorus, furnish evidence of the change from organic to inorganic phosphorus during the period of fermentation, under the conditions of this experiment.

*Changes in sulfur.* Considerable quantities of sulfur were evolved as hydrogen sulfide from the treated and untreated manure during fermentation. The formation of hydrogen sulfide, however, proceeded at a much slower rate in the untreated than in the manures treated with sulfur, calcium sulfate and acid phosphate. A measure of the hydrogen sulfide evolved was obtained by determining the sulfur content of the sodium hydroxide solutions used in the absorption of hydrogen sulfide. The weights of sulfur which were evolved as hydrogen sulfide were as follows: From manure treated with sulfur, 9.29 grams; manure treated with acid phosphate, 6.01 grams; manure

and calcium sulfate, 3.46 grams; and from the untreated manure, 0.3867 grams. These figures show that the largest amount for hydrogen sulfide was obtained from the sulfur-treated manure.

The odor of the air exhausted from the containers indicated that organic compounds were being evolved and not absorbed by the acid and alkali solutions through which the air was passed.

Determination of water-soluble sulfates in the sulfur-treated manure showed the presence of sulfates equivalent to 15.2 grams of sulfuric acid at the beginning and 38.6 grams at the end of the experiment. This is a production of sulfates equivalent to 23.4 grams of sulfuric acid during the period of fermentation. The untreated manure showed a loss of sulfates equivalent to 4 grams of sulfuric acid which is due in part to the evolution of hydrogen sulfide.

The results obtained by titrating the boiled water extracts of the sulfur-treated and untreated manure with tenth-normal alkali solution gave the same indications as were obtained by the determination of water-soluble sulfates in two samples. Water extracts of both the sulfur-treated and untreated were acid in reaction at the beginning of the experiment, while after fermentation the sulfur-treated manure had increased in acidity and the untreated manure had become alkaline in reaction.

### *Experiment with liquid manure*

The effect of sulfur, calcium sulfate and acid phosphate on liquid manure was studied under the following conditions: 500-cc. portions of cow's urine were placed in 2-quart jars and the materials referred to added in amounts as shown in table 4. The jars were covered with cheesecloth and placed in

TABLE 4

*Changes in nitrogen content of liquid manure*

| TREATMENT | WEIGHT OF NITROGEN AT BEGINNING | WEIGHT OF NITROGEN AT END | LOSS | WEIGHT OF AMMONIACAL NITROGEN AT BEGINNING | WEIGHT OF AMMONIACAL NITROGEN AT END | GAIN |
|---|---|---|---|---|---|---|
| | *grams* | *grams* | *per cent* | *grams* | *grams* | *grams* |
| 27 grams acid phosphate | 5.09 | 4.83 | 5.1 | 0.2 | 0.4642 | 0.2642 |
| 7 grams sulfur | 5.09 | 4.58 | 10.0 | 0.2 | 0.2923 | 0.0923 |
| 27 grams calcium sulfate | 5.09 | 4.595 | 9.72 | 0.2 | 3.4750 | 3.2750 |
| Untreated | 5.09 | 0.989 | 80.58 | 0.2 | 0.4299 | 0.2299 |

a room free from ammonia fumes and allowed to stand from July 8 to August 14, 1916. The contents of the jars were stirred at regular intervals during this period. The results reported showing a loss of total nitrogen and the changes in ammoniacal nitrogen occurring in the several portions are the average of duplicate determinations upon duplicate jars.

The untreated urine lost 80 per cent of its total nitrogen. While the additions of sulfur, calcium sulfate and acid phosphate have considerably decreased this loss, the results show that acid phosphate has been most effective in this respect.

The quantities of ammoniacal nitrogen found in the treated portions of urine, as compared with the ammoniacal nitrogen content of the untreated portion at the end of the experiment, afford an explanation of how the treatments have operated in preventing loss of nitrogen. In the case of urine treated with calcium sulfate, the weight of ammoniacal nitrogen at the end of the experiment was only slightly less than the total nitrogen content, indicating that ammonia was transformed into ammonium sulfate and held in this form as rapidly as it was produced.

As the ammoniacal nitrogen content of acid phosphate-treated urine at the end of the experiment is only slightly increased over that of the untreated portion, acid phosphate has prevented the formation of an appreciable amount of ammonia during the period from July 8 to August 14. The same conclusion can be drawn from the results for the sulfur-treated urine.

Some time after the period during which the experiment was continued, the formation of ammonia in the acid phosphate-treated portion developed rapidly. After the removal of a part of the contents of the jars on August 14 for determination of total and ammoniacal nitrogen the jars were sealed and allowed to stand until December 7. Upon opening the jars at this time the acid phosphate-treated sample was found to be alkaline and evolving ammonia at such a rapid rate that red litmus paper suspended in the jar was quickly changed from red to blue. This is of considerable interest in view of the fact that acid phosphate was most effective in decreasing loss of total nitrogen, and at the same time prevented the formation of ammoniacal nitrogen during the period from July 8 to August 14.

## SUMMARY

The loss of dry matter from manure after fermenting for 250 days was 32.5 per cent in untreated manure and 21.8 per cent from manures treated with acid phosphate and calcium sulfate, while the sulfur-treated manure lost 18 per cent.

Manures treated with acid phosphate, sulfur and calcium sulfate lost approximately 3.5 per cent of their total nitrogen as compared with a loss of 10.5 per cent from the untreated manure.

The water-soluble and non-protein nitrogen were greatly reduced during fermentation.

The water-soluble phosphorus has been decreased in all the samples, but at the same time the citrate-insoluble has also been decreased.

The solubility of phosphorus in 0.2 hydrochloric acid has been increased, and the organic phosphorus was greatly decreased during fermentation.

The three treated manures evolved large amounts of hydrogen sulfide. The largest amount was evolved from manure to which sulfur was added.

The manure treated with flowers of sulfur produced water-soluble sulfates equivalent to 23.4 grams of sulfuric acid, as compared with a loss of about 4 grams from the untreated sample during fermentation.

The acidity of water extracts of untreated and sulfur-treated manure was the same at the beginning of the experiment, but during fermentation the sulfur-treated manure increased in acidity, while the untreated sample became alkaline.

Sulfur, calcium sulfate and acid phosphate were very effective in preventing loss of nitrogen from urine. The untreated urine lost 80 per cent of its total nitrogen.

Treatment with sulfur reduced the loss of nitrogen to 10 per cent and prevented formation of ammonium salts.

The calcium sulfate-treated sample lost 9.7 per cent of its nitrogen, and 68 per cent of its total nitrogen was transformed to ammonium sulfate and held as such.

The urine treated with acid phosphate lost only 5 per cent of its nitrogen, and the treatment prevented the formation of ammoniacal nitrogen in an open container during the 37-day period of the experiment.

After standing in a closed jar three months longer, the acid phosphate-treated urine was found to be alkaline and evolving ammonia.

## REFERENCES

(1) Berry, R. A. 1914 The results of some experiments with barnyard manure. West of Scot. Agr. Col. Bul. 65.

(2) Bjorn-Anderson, H. 1905 The loss of nitrogen from liquid manure and its prevention by superphosphate. *In* Tidsskr. Landökonom., 1905, no. 3, pp. 160–168.

(3) Bohme, E. 1905 Preservation and action of the nitrogen of urine. *In* Centbl. Agr. Chem., Bd. 34, no. 5, p. 300.

(4) Collison, R. C. 1912 Inorganic phosphorus in plant substances—a method of estimation. *In* Jour. Indus. Engin. Chem., v. 4, no. 8, p. 606.

(5) Cook, F. C., and Hutchinson, P. H. 1915 Experiments during 1915 in the destruction of fly larvæ in horse manure. U. S. Dept. Agr. Bul. 408.

(6) Copenhagen Station Results 1906 The storage of liquid manure. *Abs. in* Exp. Sta. Rec., v. 8, p. 618.

(7) Deherain, P. P., and Dupont, C. 1901 On the fermentation of the nitrogenous matter of manure. *In* Ann. Agron., t. 27, no. 9, pp. 401–427.

(8) Dietzell, B. E. 1897 Experiments on the preservation of barnyard manure. *In* Landw. Vers. Stat., Bd. 48, No. 3–5, p. 163.

(9) Gerlach. 1915 The action of gaseous ammonia on superphosphates. *In* Chem. Abs., v. 10, no. 8, p. 1071.

(10) Hall, A. D. 1909 Fertilisers and Manures. London, p. 186.

(11) Lipman, J. G., McLean, H. C., and Lint, H. C. 1916 The oxidation of sulfur in soils as a means of increasing the availability of mineral phosphates. *In* Soil Sci., v. 1, no. 6, p. 533.

(12) McBeth, I. G. 1916 Studies on the decomposition of cellulose in soils. *In* Soil Sci., v. 1, no. 5, p. 437.

(13) Niklewski, Von Bronislaw 1910 Conditions of nitrification in stall manure. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 26, p. 388.

(14) Pfeiffer, T. 1897 Contributions to the subject of the changes occurring in the decomposition of nitrogenous organic substances. *In* Landw. Vers. Stat., Bd. 48, No. 3–5, p. 189.

(15) Schneidewind, W. 1907 To reduce the loss of nitrogen in barnyard manure. *In* Landw. Jahrb., Bd. 36, no. 4, p. 569.

(16) Severin, S. A. 1903 Gypsum for fixing ammonia in decomposing manure. *In* Jour. Expt. Landw., Bd. 4, no. 1, p. 88.

(17) Sjollema, B., and Ruyter de Wildt, J. C. de 1908. Versuche über Zersetzungen im Stallmist und über die Wirkung desselben. *In* Vers. Landbouwk. Onderzoels. Rykslandbouwproefstat., 1907, no. 1, p. 21. Abs. *in* Centbl. Agr. Chem., Bd. 37, no. 10, p. 652.

(18) Stoklasa, J. 1894 The losses of nitrogen in barnyard manure and their prevention. *In* Oesterr. Ungar. Ztschr. Zuckerindus. u. Landw., Bd. 23, p. 525–536. *Abs. in* Chem. Centbl., Bd. 45, Abt. 2, no. 19, p. 800.

(19) Street, J. P. 1900 The reduction of nitrates in the presence of barnyard manure. *In* N. J. Agr. Exp. Sta. 21st. Ann. Rpt. [1899–1900], p. 87.

(20) Tottingham, W. E., and Hoffmann C. 1913 Nature of changes in solubility and availability of phosphorus in fermenting mixtures. Wis. Agr. Exp. Sta. Research Bul. 29.

(21) Wagner, P. 1897 The fertilizing value and the preservation of barnyard manure. *In* Landw. Vers. Stat., Bd. 48, no. 3–5, p. 360.